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THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXXXIII—No. 2

Issued January 1, 1928

BALTIMORE, U. S. A.

1928

tered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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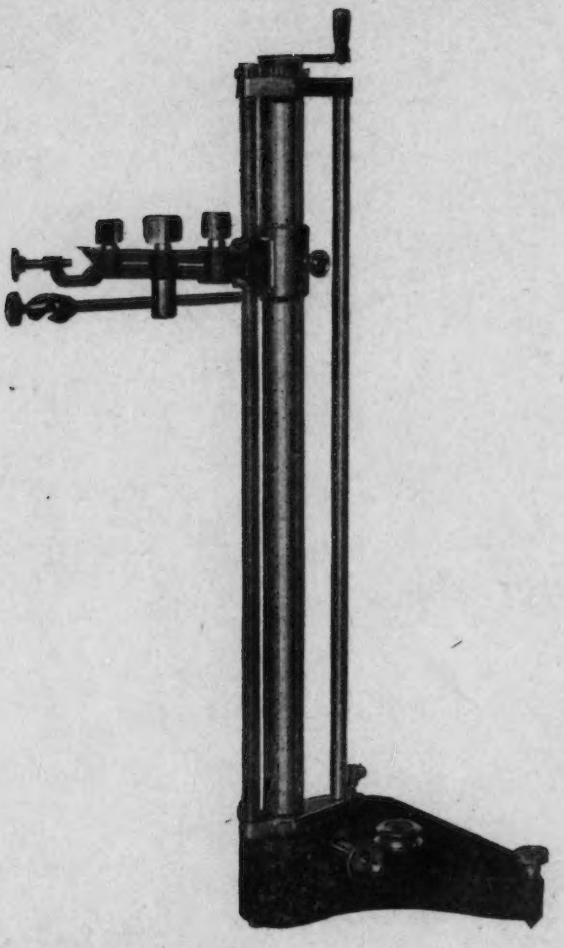

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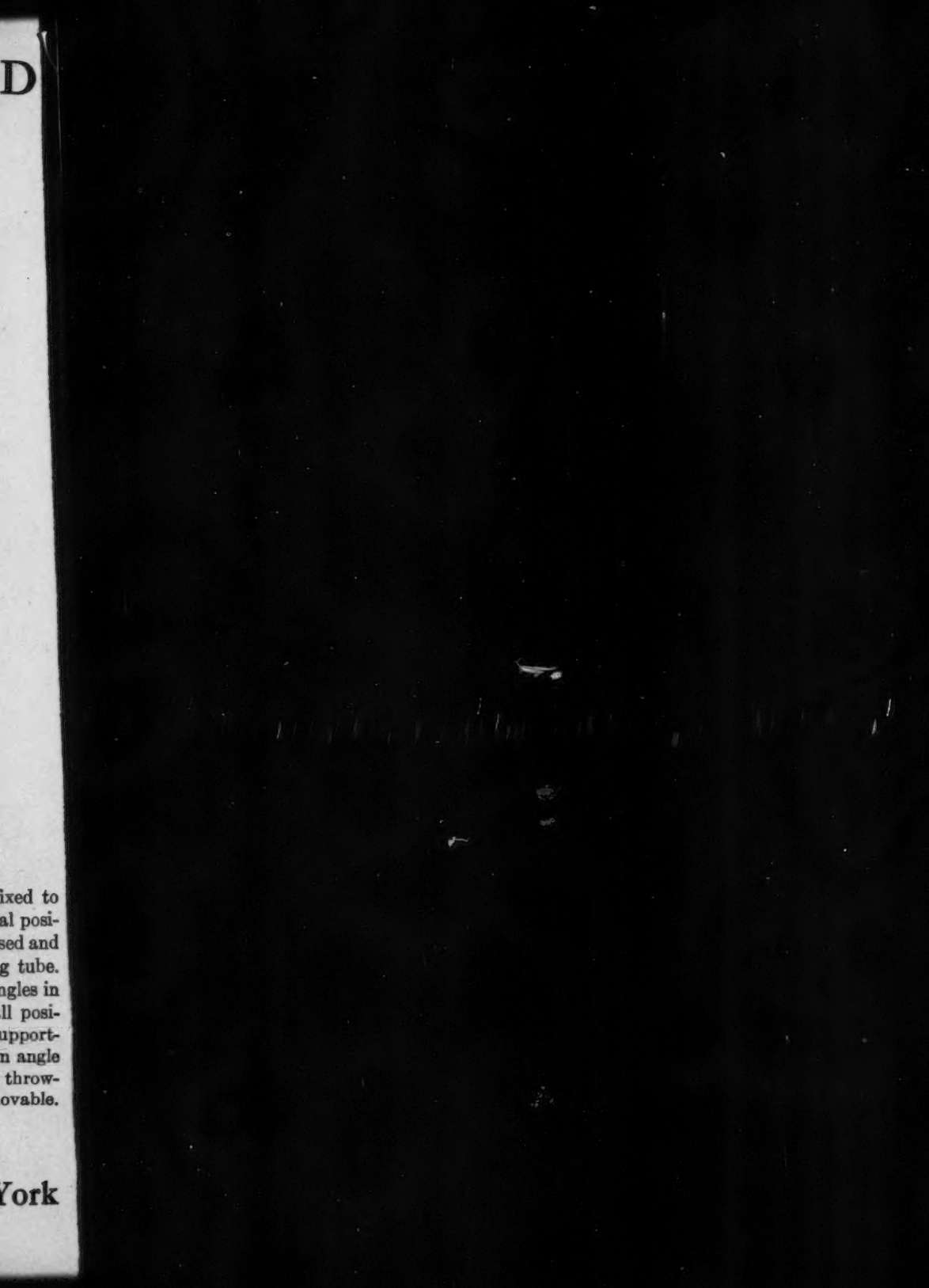

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 83 JANUARY 1, 1928 No. 2

STUDIES IN ACTIVITY

III. The Influence of Phosphate Feeding on Activity; The Relation of Blood Sugar to Activity

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From the Department of Psychology, University of Minnesota

Received for publication August 23, 1927

Increased activity after phosphate feeding. *1. Review of the literature.* That phosphates furnish a stimulus to activity appears from the general trend of a series of experiments by Embden. When he analyzed the juice from muscle which had been treated with sodium bicarbonate at 40°C. in order to allow the maximum amount of lactic acid to develop, he found equal molecular concentrations of lactic acid and phosphoric acid (Embden, 1914). He had formerly held that the lactic acid, produced either by muscular contraction or by heat rigor, was present in the muscle in a precursor form, which he named lactacidogen. Glucose was known to be the heat producing part of the lactacidogen molecule. His discovery of phosphoric acid along with lactic acid as the end products of muscular work led him to conclude that lactacidogen was a hexosephosphate linkage. The splitting of lactacidogen into lactic acid and phosphoric acid he calls dissimilation; its synthesis from carbohydrate and phosphates he calls assimilation.

After further work on the dissimilation process, Embden turned his attention to the assimilation process. He confirmed his hypothesis that the feeding of sugar and phosphates should increase the lactacidogen content of muscle by experiments on dogs and rabbits. Furthermore he found a decrease in lactacidogen after giving phloridzin, and after exercise induced by shivering or by strychnine (Embden, 1921 a).

The feeding of phosphates to soldiers yielded an encouraging increase in their endurance (Embden, 1921 b). In his preliminary work he had the

subject drink 250 cc. of 4 per cent sugar solution containing 1 per cent NaH_2PO_4 a day. In later work he increased the phosphate dosage from 2.5 grams to 7.5 grams a day. On control days the subject drank 4 per cent sugar water containing 1 per cent tartaric acid. The subject's endurance was tested by one hour's work in a treadmill. On phosphate days the amount of work done was appreciably greater than on control days. In most cases the greatest effect of the phosphate was noticed on the day after its consumption, but the effect of the dose was usually noticed within 6 hours after feeding. One subject complained of a sleepless night when the phosphate was taken in the evening. Phosphate feeding was then tried out on soldiers on the march. Each man received 7.5 grams per day. Not only were the phosphate-fed troops fresher than control troops in the same regiment, after a long day's march, but they were also more alert when at rest. Some restlessness at night was noticed among them. But the greatest difference between the two groups, according to Embden, was psychic. Whereas the control-fed soldiers were dumb from fatigue after a day's advance, the phosphate-fed men shouted and sang gayly. Some phosphate feeding was done on horses, the dosage being 80 grams a day; favorable results were noted.

The work of Elias and Weiss (1922), on injecting phosphate into the blood of diabetics, also bears on this general topic. Intravenous injection of a hypertonic solution of either mono- or di-basic sodium phosphate was followed by a fall in sugar in diabetic or alimentary hyperglycemia. No effect was noted on the normal individual. The duration of the fall was about 24 hours; urine sugar remained low for 3 days. Dibasic sodium phosphate they found to be three times as potent as monobasic, since N/3 Na_2HPO_4 gave the same decrease in sugar level as an N NaH_2PO_4. They believe that the phosphate has a direct point of attack on the blood, but only an indirect and secondary attack on the kidney. They fail to find evidence, however, that the phosphate is synthesized into hexosediphosphate since no more carbohydrate is found in the blood than occurs normally.

Other studies on the relation of phosphates to carbohydrate metabolism may throw some light for the theoretical interpretation of the results about to be presented. Sokhey and Allan (1924) report that in normal dogs the ingestion of sugar, or the administration of insulin, or both together increases the output of phosphorous in the urine by about 25 per cent. In diabetic dogs not under the influence of insulin, phosphate excretion is almost double that of normals. Sugar ingestion does not affect this high level of excretion, but insulin dosage brings it down to the level for normal dogs. A change in phosphate excretion similar to that caused by insulin also occurs after injection of adrenalin according to Allan (1924). Phloridzin induces an excessive loss of phosphates. These two papers point to a

phosphate sparing action accompanying the combustion of carbohydrates.

Since both glucose and insulin cause a rise in phosphate excretion, though the action of glucose is somewhat slower than insulin, Markowitz (1925) hypothecates that glucose merely stimulates insulin production and that insulin causes the phosphate rise. This hypothesis is proved by the fact that depancreatized dogs, which have no insulin, excrete no more phosphates upon ingestion of glucose than they normally do. He suggests that insulin may act as the hexosephosphoric acid synthesizer.

Kay and Robinson (1924) offer evidence for the last hypothesis. After insulin administration they find that organic phosphoric esters, which probably contain a sugar radical, are synthesized from the inorganic phosphates of the blood. If it is sugar which is linked with the phosphate, 40 per cent of the sugar disappearing from the blood would thus be accounted for. In muscle there is a 50 per cent increase in phosphoric ester, or lactacidogen phosphorus as Embden would call it. If the hexose which goes to form this hexosephosphate or lactacidogen came from blood sugar, it would account for 7 times the amount which disappeared from the blood upon insulin injection.

2. *Method.* In connection with other work on activity an attempt was made to verify Embden's findings that phosphate feeding makes for greater motor efficiency. Three different experiments were run in which 30, 28 and 39 male rats participated. Their activity was measured in revolving drums.[1] After the animals had been in the cages for a sufficient length of time to have become thoroughly accustomed to them their activity was recorded for a preliminary period of 5 days and for a final 5 days, which served as control periods for the intervening period of phosphate feeding. Between these two control periods a 10-day period of phosphate feeding was introduced. On phosphate days 2 cc. of a 5 per cent solution of dibasic sodium phosphate (Na_2HPO_4) were mixed with the 30 cc. of bread and milk which was the ration of food allowed for each rat. Thus each animal received 0.1 gram of phosphate a day. Relative to body weight this dosage is about 5 times as heavy as Embden's 7.5 grams for a man.

3. *Results.* In the first experiment the animals showed decided gains during the phosphate feeding. In the second experiment, on the other hand, there was a slight loss in activity under phosphate. In the third experiment, again, the rats gained on the average. In two out of three experiments the results lie in the same direction. The results are tabulated below.

The superiority of the last 5 phosphate days over the 5 succeeding control days is slightly greater than that of the first 5 phosphate days over the five preceding control days. This would seem to indicate that the effect

[1] For more complete description of the method see article I of this series.

of phosphate feeding is cumulative. Embden found the same thing in his experiment on humans. The gains are all small, and the large variability makes the differences which do appear non-significant according to the formula D/PE_D. The consistency with which the results do turn out in the same direction upon repetition of the experiment gives them somewhat more weight. Under normal conditions the chances are equal that a rat will gain or will lose from one 5-day period to the next. Actual count gives 50 per cent losing and 50 per cent gaining. In the phosphate experiment, however, only 22 out of 97 animals lost.

TABLE 18[2]

Showing averages under phosphate feeding

	MEAN DAILY REVOLUTIONS			
	Group I—30 Cases	Group II—28 Cases	Group III—39 Cases	Total 97 Cases
Days 1–5 control	3347 ±509	3493 ±548	5380 ±473	4238 ±300
Days 1–5 phosphate	5420 ±783	2885 ±416	5743 ±465	4818 ±339
Gain under P	2073 ±934	−608 ±681	263 ±663	580 ±453
Days 6–10 control	4671 ±591	2567 ±384	4290 ±343	3910 ±259
Days 6–10 phosphate	5944 ±861	2682 ±371	5796 ±455	4943 ±354
Gain under phosphate	2273 ±1045	115 ±534	1506 ±570	1033 ±439
10 control	4023 ±888	2953 ±386	4743 ±520	4007 ±332
10 phosphate	5630 ±818	2757 ±379	5580 ±394	4781 ±402
Gain under phosphate	1607 ±1207	−198 ±541	837 ±652	774 ±521

Percent gains were determined for each rat. The average of these percents were as follows:

Group I	32 per cent
Group II	10 per cent
Group III	24 per cent
Total 97 cases	22 per cent

4. *Discussion.* As Embden has reported, the effect of phosphate feeding is to increase muscular efficiency by a slight amount. It seems that the value of phosphate feeding lies in the fact that it furnishes a supply of the phosphate in a readily available form for synthesis with glucose into hexosephosphate or lactacidogen. Many of the phosphates of the body which are normally built up with hexoses into lactacidogen are in organic compounds, such as in lecithin and nucleoproteins. A large part of the reserve phosphates are stored in the bones. Hence, although the phosphate supply of the body is probably adequate to meet the demands of heavy muscular work, it can be assembled only by the breaking down of other compounds. Phosphate which is fed is ready for immediate use. The bread and milk

[2] Tables are numbered in continuation from article II of this series.

diet of these rats is one containing a large amount of phosphate. It is particularly interesting that even on this diet most rats showed increased activity.

BLOOD SUGAR AND ACTIVITY. The energy for muscular contraction comes from the oxidation of glucose. This fact would point to an intimate relation between carbohydrate metabolism and activity. There is no doubt that an animal which runs 20,000 revolutions a day burns more sugar in the process than does an animal which runs 200 revolutions. But whether or not a low level of sugar in the blood may be a stimulus to activity is a matter for investigation. The muscular weakness and lethargy of the diabetic might suggest that a low level of activity accompanies a high level of blood sugar. High sugar content cannot in itself be looked upon as the cause of this lethargy. It is rather the diabetic's inability to burn sugar, together with his oxidation of body proteins which makes him weak.

The problem which I have attempted to get at in this work is the normal relation between blood sugar level and spontaneous activity. It will be recalled that the normal sugar content of human blood is between 80 and 120 mgm. per 100 cc. That for other mammals is about the same. Within this range there is room for a good deal of differentiation.

1. *Method.* The rats used for the blood sugar work had run in the activity cages more than two months. The median of their last 5 days' runs was taken as the activity measure to be correlated with blood sugars. The rats received their last food 24 hours before they were killed, but they remained in the activity cages till a short time before their death. Care was taken not to excite the animal before taking his blood. He was beheaded by a quick blow from a cleaver, and the blood was caught in a small beaker, on which a drop of potassium oxalate solution had been dried to prevent clotting of the blood.

Folin's method for preparing the blood filtrate and for determining the sugar was used, his recent precautions with regard to the copper solution, and the neutrality of the filtrate (Folin 1926 a, b) were taken.

2. *Results.* All the sugars obtained were within the normal range. The lowest was 68, the highest 125, and the average 95 mgm. per 100 cc. of blood. Nineteen of the rats were killed about two months earlier than the others. In running correlations the two groups were treated separately as well as together. Correlations between activity and blood sugar are given below.[3]

Group I, 19 cases, blood sugar and activity	-0.21 ± 0.155
Group II, 26 cases, blood sugar and activity	-0.05 ± 0.139
All animals, 45 cases, blood sugar and activity	-0.21 ± 0.10

[3] Since the above was written blood sugars have been done on a third group of 17 animals. This work yielded a correlation of -0.19 between activity and blood sugar.

The correlations here are so low as to be insignificant in themselves. However, they are consistent as to direction, and two of them are of the same general magnitude. One factor which might have worked to reduce the correlation and thus to obscure any true relationship which might have existed is the fact that the sugars vary over a narrow range, whereas the variations in activity were large. Three milligrams' difference in sugar content sometimes made a difference of 8 points in rank; it is improbable that such minute differences in sugar content would show up equally in activity even if the relation between sugar level and activity were well nigh perfect.

Consequently the extremes of the sugar distribution were picked out and corresponding activity records computed. Nineteen animals had sugar determinations of 100 mgm. and above; 6 animals had sugars under 90. The average activity for the two groups is:

	AVERAGE SUGAR	ACTIVITY (REVOLUTIONS PER DAY)
	mgm.	
High sugar group	112	3468 ± 268
Low sugar group	82	5749 ± 773

$$D/PE_D = 2281/818 = 2.8$$

The difference in activity between the two groups is pretty large, and although it is not a statistically significant difference, yet there are about 97 chances in 100 that a similar difference will occur again.

This work can by no means be considered conclusive. It does, however, open up an interesting lead for more work. The tendency for low blood sugar to accompany high activity is certainly more than hinted at. The most plausible explanation for this lower sugar is that the more active animals are using up their sugar supply at a faster rate than the inactive animals. There is a possibility, too, that a low sugar level is another "drive" to activity. The hours just after feeding are hours of little activity; (Shirley, 1928) they are also the hours of alimentary hyperglycemia. But to draw any other inferences or conclusions from these data than that blood sugar bears a slightly negative relation to activity would be unwarranted. The results merely indicate that in the search for factors underlying individual differences in activity, analysis of blood sugar will not lead only into a blind alley.

SUMMARY

1. Phosphate feeding acts as a mild stimulant to spontaneous activity. The results of this investigation are consistent with those of other experimenters.

2. The relation between blood sugar and activity seems slightly negative.

BIBLIOGRAPHY

ALLAN, DICKSON AND MARKOWITZ. 1924. This Journal, lxx, 333.
ELIAS, H. UND WEISS. 1922. Wien. Arch. f. inn. Med., iv, 29.
EMBDEN, GRIESBACH UND SCHMITZ. 1914. Zeitschr. f. Physiol. Chem., xciii, 1.
EMBDEN, SCHMITZ UND MEINCHE. 1921a. Zeitschr. f. Physiol. Chem., cxiii, 12.
EMBDEN, GRAFE UND SCHMITZ. 1921b. Zeitschr. f. Physiol. Chem., cxiii, 67.
FOLIN, O. 1926a. Journ. Biol. Chem., lxvii, 357.
FOLIN, O. AND A. SVEDBERG. 1926b. Journ. Biol. Chem., lxx, 405.
KAY, H. D. AND R. ROBINSON. 1924. Biochem. Journ., xviii, 1139.
MARKOWITZ, J. 1925. This Journal, lxxvi, 524.
SHIRLEY, M. 1928. Journ. Comp. Psych., viii.
SOKHEY, S. S. AND F. N. ALLAN. 1924. Biochem. Journ., xviii, 1170.

THE RESPIRATORY RESPONSE TO CARBON DIOXIDE

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Received for publication September 21, 1927

As is well known, when air containing an increased percentage of carbon dioxide is breathed the volume of respiration is increased. However, that the maximum increase in respiration occurs only after the experiment has continued for some time does not appear to be definitely recognized. A review of the literature on the respiratory response to carbon dioxide shows only scant allusion to the existence of this "lag" phenomenon, and, indeed, this subject is based on rebreathing experiments in which, of course, no opportunity is afforded to observe a "lag." The present paper deals with this "lag" in the respiratory response to carbon dioxide.

Douglas and Haldane (1909) in some brief experiments on man breathing 7.18 per cent and 4.74 per cent carbon dioxide from a Douglas bag, found that about thirty respirations were required for the hyperpnea to reach a maximum. When breathing the 4.74 per cent mixture, an alveolar air sample taken at the fifth respiration contained 6.82 per cent and at the thirtieth 6.56 per cent carbon dioxide. Lindhard (1911) discussing experiments on breathing carbon dioxide mixtures from a 150 liter spirometer states, "The experiment begins as soon as he has adapted himself to the air mixture present; this will always be the case after some 20 liters have been inspired." Scott (1920) studied two normal individuals breathing carbon dioxide mixtures from a 240 liter spirometer, and reported that the subject must breath 50 to 60 liters of the mixture to accommodate himself to the new conditions. With a 6 per cent mixture, however, the whole experiment lasted only 3 minutes. de Almeida (1923), describing breathing from a spirometer containing known mixtures, observes "Five minutes must be allowed before making observations in order to allow respiration to accommodate itself to the new conditions." This observation is more in accord with the results presented in this paper than any of the previous ones; although as will be seen later, it does not tell the whole story.

Where breathing is carried out from a closed system, the carbon dioxide percentage of the inspired air is variable and continually increasing. Under these conditions there is no opportunity to observe the "lag," but the question at once arises whether or not the response obtained is maximal. One would expect results to vary with the rapidity of increase of the

carbon dioxide, which would depend on the volume of air in the rebreathing system. Hough (1910, 1911) found in rebreathing experiments no difference in the response of the same individual when 22.5, 30 or 40 liters were rebreathed. Peabody (1915) carried out rebreathing experiments on nine normal subjects, using a system which we estimate to have contained about 40 liters. He termed the increased response to carbon dioxide in terms of the normal respiratory volume taken as 100, the ventilation coefficient, and gives curves relating this to the carbon dioxide content of the inspired air. Davies, Brow and Binger (1925) did a careful series of experiments upon one individual using a rebreathing system containing about 100 liters. They proposed the formula $Y = K + ab^x$, where Y is the ventilation coefficient, X is the percentage of carbon dioxide in the inspired air, and K, a and b are constants for the individual. Observed results corresponded very closely to results calculated by this formula up to about 5 per cent carbon dioxide in the inspired air, but there was some variation above this level, the calculated result in each case being higher than the observed one. The responses which they obtained with their one subject were always greater than any of those obtained by Scott or Peabody.

Methods. Using cylinders of compressed commercial carbon dioxide and of compressed air, mixtures of carbon dioxide in air were made up in 250 cu. ft. gas cylinders (under about 1000 pounds' pressure) according to the method used for some years in this laboratory. The mixtures were analysed for carbon dioxide in triplicate with the Henderson-Haldane apparatus. The oxygen percentage was not considered, since even with the highest concentration of carbon dioxide used it was only slightly lowered.

In doing the experiments a cylinder of a mixture was connected through a reducing valve to the side tube of a 30 liter Douglas bag, which served as a reservoir. The main exit of the bag led through a large bore three-way tap to the inspiratory side of a respiratory valve. The tap was so arranged that inspiration might be made either from the bag or from room air. The respiratory valve was equipped with the usual rubber mouthpiece, and as used increased the personal dead space 60 cc. Its expiratory side was connected to a three-way tap, so that expiration might be directed either through a meter or through a Haldane alveolar air tube, equipped at the distal end with a Sadd flutter valve.

The meter was a B. McDonald & Company 20 light "B" type (dry). Its accuracy was tested by breathing through it at various rates, collecting the expired air in a Douglas bag, and measuring it through a wet meter. It was found to have an average error of $\pm$ 1.7 per cent for rates of 5 to 80 liters per minute, with a maximum error of + 3.0 per cent at 58 liters per minute. Since this was well within the variation in observations, it could be neglected.

All connections were made with one inch non-collapsible tubing, and were as short as possible.

In the rebreathing experiments the expiratory side of the respiratory valve led through the meter to a three-way tap which would direct the expired air either into the room or into the spirometer. The inspiratory side of the respiratory valve was connected to a three-way tap, so that inspiration could be from the room or the spirometer, and a side tube for collecting samples of the inspired air was interposed near the tap.

Two spirometers were used; a small Krogh basal metabolism type with the soda-lime container removed, which gave the system a capacity of 25 liters, and a large, eccentrically counterbalanced Tissot, which as used gave the system a capacity of 85 liters. The latter was equipped in the top of the bell with a mixing fan, driven through a water seal by a small electric motor, which insured adequate mixing of the gases within. Neither spirometer gave any subjective sense of resistance, even at the highest respiratory minute volumes.

The subjects for the experiments were male medical students, about 25 years of age, all of whom had previously had experience breathing through respiratory valves.

The experiments were done either in the late morning or early afternoon. The subjects came to the laboratory with no previous preparation (except as indicated in the results), sat in a comfortable chair before the apparatus for 15 to 30 minutes and then inserted the mouthpiece and adjusted a noseclip. The subject was then allowed to breathe room air until the respiratory minute volume had become constant for three minutes or more. This usually required 5 to 10 minutes.

In making the observations a stop watch with a split second hand was employed. By this means the time could be accurately taken and the meter read on the end of an expiration at the end of every minute throughout the experiment. After the respiratory minute volume had become constant, the three-way tap allowing inspiration from the bag was turned, and the observations continued. The mixture from the cylinder was run into the bag as needed by regulating the reducing valve. The experiment was allowed to run about ten minutes, which was as long as the higher percentages of carbon dioxide could be breathed without discomfort. The subject was then turned back to air and records of the disappearance of the hyperpnea made in the same fashion.

In computing the results, the difference between successive readings of the meter could be reduced to liters per minute on the basis of the readings of the stopwatch.

In the alveolar air experiments the expired air was allowed to go through the meter for one minute, at the end of which the tap was turned so that expiration was through the Haldane tube. In the middle of this minute

a forced expiration was made at the end of a normal expiration (Bock and Field, 1924–1925) and the tap was immediately turned back. A sample of the alveolar air was immediately taken over mercury, the Sadd valve having trapped the gas in the tube. The reading of the meter was neglected during the rest of that minute, but readings were made at the beginning and end of the next minute. In this fashion alveolar air samples could be taken every other minute during an experiment, the alternate minutes being used to observe the respiratory minute volume.

In the rebreathing experiments the spirometers were filled with oxygen, Davies, Brow and Binger (1925) having shown that there is probably little difference in reaction to carbon dioxide in the presence of high concentrations of oxygen. After a satisfactory normal was obtained in the usual

TABLE 1

SUBJECT	1.60 PER CENT CO_2			3.05 PER CENT CO_2			4.55 PER CENT CO_2			5.96 PER CENT CO_2		
	Lag	Average response to mixture	Response, rebreathing	Lag	Average response to mixture	Response, rebreathing	Lag	Average response to mixture	Response, rebreathing	Lag	Average response to mixture	Response, rebreathing
	minutes	*liters per minute*	*liters per minute*	*minutes*	*liters per minute*	*liters per minute*	*minutes*	*liters per minute*	*liters per minute*	*minutes*	*liters per minute*	*liters per minute*
J. N. B.....	2	12.0	11.1	2	16.2	13.4	4	22.2	15.7	6	26.6	20.7
M. B. K.....	0	9.6		5	12.9		6	21.4		6	35.3	
N. A. P.....	3	11.3	11.4	3	13.9	14.4	4	22.0	21.8	7	48.2	37.7
F. G. S.....	2	10.1	10.0	3	12.5	12.2	5	18.7	19.0	7	24.3	23.7
E. K. M., Jr...				4	13.6	13.0	5	17.9	18.3	7	24.3	22.9
Average.....	1.8	10.8	10.8	3.4	13.8	13.3	4.8	20.4	19.0	6.6	31.7	26.3

way, both taps were turned simultaneously at the end of an expiration, so that respiration was to and from the spirometer.

Using the small spirometer observations were made in the same way as when breathing mixtures, and in the middle of the last minute of the experiment a sample was taken from the intake for analysis. The experiments were allowed to proceed 9 or 10 minutes, since this period was found to build up about 6 per cent carbon dioxide in the system.

When employing the large spirometer time was taken and the meter read every three minutes, and a sample was taken from the intake for analysis in the middle of each three minute period.

All gas samples were taken over mercury and were analysed, usually in duplicate, with the Henderson-Haldane apparatus.

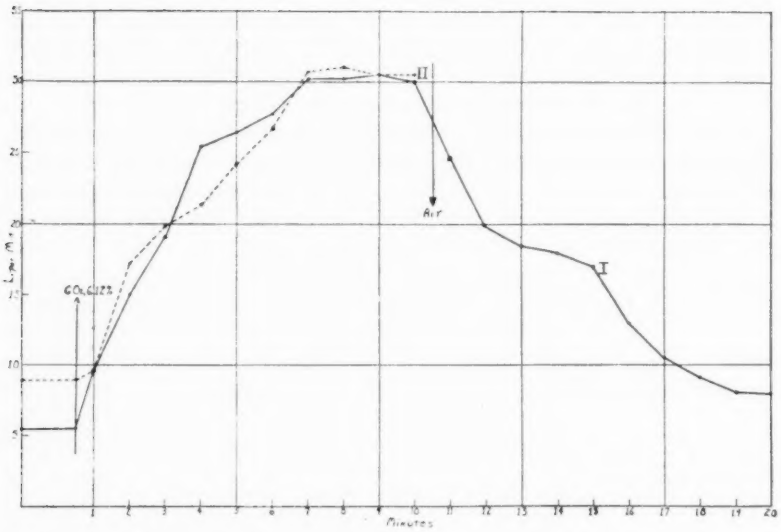

Fig. 1. The effect of the normal on reaction. Curve *I* was made under basal conditions, and curve *II* was made 25 minutes later, and 15 minutes after food and exercise. The variations are small enough to be neglected. Curve *I* shows as well that the "lag" in the disappearance of the hyperpnea adopts the same form as the "lag" in its appearance.

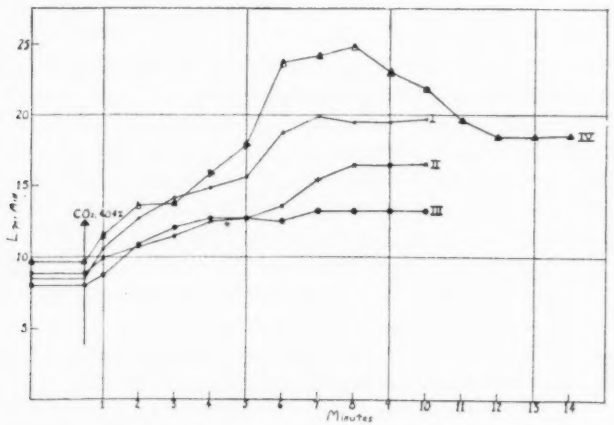

Fig. 2. Individual variation in reaction to 4.04 per cent CO_2. Curve *I* shows the maximum and curve *III* the minimum reaction to 4.04 per cent CO_2 met with in sixteen individuals. Curve *IV* shows the variation, met with in three individuals in the series.

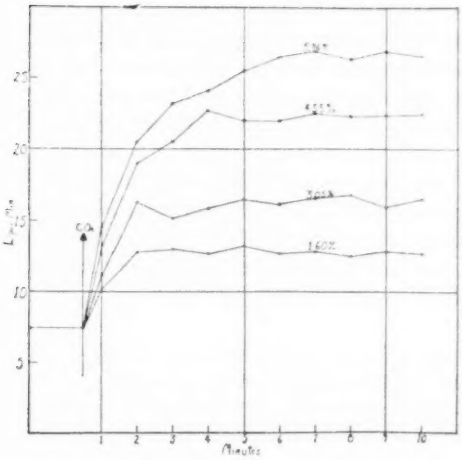

Fig. 3. The reaction of one individual to four percentages of CO_2. The length of the "lag" and the height of the response are in direct relation to the concentration of CO_2 being inspired.

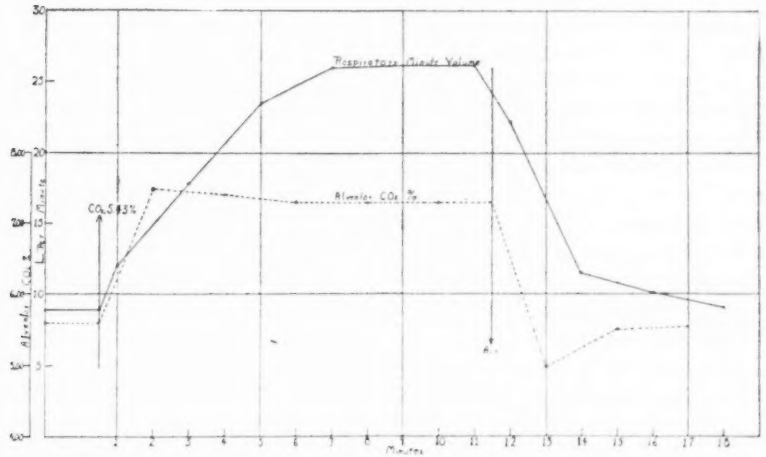

Fig. 4. Comparison of respiratory minute volume and alveolar CO_2 per cent. The alveolar CO_2 rises at once and drops slightly as the hyperpnea is being established, and drops below the normal to rise to it again as the hyperpnea disappears.

RESULTS. The following results are selected from a series of 74 experiments made upon 20 individuals. No attempt is made to present all of the data; typical experiments which are illustrative have been chosen, and such variations from the typical as have been noted are indicated.

For convenience in discussion the results are considered under six headings, the data being presented graphically or by means of tables. The tables are self-explanatory, and in the graphs the average normal is given as a straight line, the heavy vertical lines mark the beginning or end of the stimulus, and time is plotted as the midpoint of the minutes after the beginning of the stimulus.

1. The "lag" phenomenon. From the outset in the experiments the existence of a "lag" in the respiratory response was demonstrated. Figures 1, 2, 3 and 4 demonstrate this fact, and table 1 shows that there is individual variation in the "lag", and that it is longer for the higher percentages. Figure 1 shows as well that there is a lag in the disappearance of the hyperpnea.

2. The constancy of individual reaction. Hough (1910, 1911) had observed in rebreathing experiments that the reaction of one individual was constant upon different occasions, but Davies, Brow and Binger (1925) had concluded that the reactions of one individual must vary on different days. In our experiments of 29 repeated observations on 10 individuals on different days, all but two agreed perfectly in their reaction to the same percentage of carbon dioxide. Of these two examples of disagreement, one was thought due to the fact that the subject was relatively untrained, but the other instance was upon a trained subject, and one felt that the disagreement was an actuality. No effort was made to seek an explanation for this phenomenon.

Davies, Brow and Binger (1925) had had carefully established normals of basal or nearly basal level upon which to begin their experiments, but we were working under conditions in which this was not feasible. Accordingly we were much interested in the effect of the level of the normal upon the reaction to carbon dioxide, and experiments were made upon four individuals to determine this. In each case two experiments using the same gas mixture were made upon the same individual on the same day. In one experiment the conditions would be basal or nearly so, while the other experiment would be done after food, exercise, or some other supposed disturbing influence.

Figure 1 shows the results of such an experiment. Curve *I* was made under basal conditions, while curve *II* was made 25 minutes later, and 15 minutes after the subject had walked down and up two flights of stairs and had drunk a cup of coffee. The other experiments gave the same results.

3. Individual variation. All of the authors have agreed that there is an individual variation in respiratory response to the same stimulus. Figure

2 substantiates this idea by comparing the reactions of four individuals to 4.04 per cent carbon dioxide. Curves *I*, *II* and *III* show the typical reaction, *I* being the maximum and *III* the minimum response met with in sixteen individuals, while *IV* shows a variation, met with three times in twenty individuals, of a rise, a later fall, and the establishment of the plateau on a much lower level. This phenomenon was thought at first to be psychic, but repeated observations on W. B. H., a trained subject, left one feeling that it was not.

Table 1 shows the difference in the height of the response and the length of the lag on five individuals, using four different mixtures.

4. The response to different concentrations of carbon dioxide. Five individuals were studied with a series of four mixtures; 1.60 per cent, 3.05 per cent, 4.55 per cent and 5.96 per cent. On each subject the series of four was done at one time, allowing only enough time between experiments for recovery from the previous one, which was usually about half an hour.

TABLE 2

SUBJECT	CO_2 ATTAINED REBREATHING	RESPIRATORY MINUTE VOLUME REBREATHING (LAST MINUTE)	AVERAGE RESPONSE TO 6.12 PER CENT CO_2
	per cent		*liters per minute*
W. W. N.	5.74	21.0	30.2
W. B. H.	6.22	19.3	29.3
H. L.	6.09	22.0	29.0
M. B. P.	6.00	20.8	25.3

Figure 3 shows a typical result, and table 1 shows the time of the lag and the average response on the plateau of the curve.

5. Rebreathing experiments. The rebreathing experiments in the literature seemed to fall into two groups; those employing a small volume, as did Hough (1910, 1911) Peabody (1905) and others and the experiments of Davies, Brow and Binger (1925), using a large volume. Accordingly, two series of experiments were done.

Using the small spirometer, table 2 shows the percent of carbon dioxide attained, the respiratory minute volume during the last minute of the experiment, and the average maximum response to 6.12 per cent carbon dioxide of that individual, done in each case either just before or just after the rebreathing experiment. It will be seen that in each case the results of the rebreathing are lower than the response to 6.12 per cent carbon dioxide, though the concentration of carbon dioxide attained in the spirometer was in every case about 6 per cent.

When the large spirometer was used, the differences were not so marked. Table 1 compares the average response to four mixtures for four indi-

viduals and the response for the same concentration of carbon dioxide while rebreathing a large volume. The figures for rebreathing were read off curves plotted for the experiments, each curve having eight or nine points and the same general shape as the curves of Davies, Brow and Binger (1925) when these latter are plotted as liters per minute instead of percentile increase of the basal respiratory minute volume.

It will be seen that the agreement is fairly good; two of the experiments show absolute agreement, but the other two are somewhat at variance, especially at the higher levels.

6. Alveolar air samples. Five experiments were carried out in which alveolar air samples were taken during the course of the experiment. Samples were taken only at the end of a normal expiration (Bock and Field, 1924–1925). In three of the experiments a mixture containing 5.96 per cent carbon dioxide was used while in the other two, 5.43 per cent was breathed. A regular response curve done just before or after the alveolar air experiment, indicated that the disturbance occasioned by the forced expirations did not appreciably alter the curve of response to the mixture. Figure 4 gives the result of a typical experiment. It is seen that the alveolar carbon dioxide rises immediately and falls slightly as the hyperpnea develops, and on the return to breathing air falls below the normal level and then rises to it as the hyperpnea disappears. The other four experiments confirmed this result.

Discussion. The demonstration of the "lag" in the response of the respiration to breathing increased concentrations of carbon dioxide indicates clearly that an experiment must be carried out for a considerable time to obtain the true maximal response to any mixture. Results which do not take this phenomenon into account will vary with the time at which the response is taken, and will also be lower than the true maximal response.

That the level of the normal has little or no effect on the extent or nature of the reaction is indicated, and if further experiments prove this to be true it will make the scheme proposed by Peabody (1915) of plotting the respiratory response in terms of the percentile increase of the normal, artificial, and will warrant conclusions that the extent of respiratory response to a given concentration of carbon dioxide is a factor determined, under given conditions, only by the strength of the stimulus.

Immediately upon demonstration of the "lag" the question arose whether rebreathing experiments could show the maximum response to the higher percentages of carbon dioxide. This depended, we thought upon whether sufficient time were consumed in building up the higher percentages to allow for the "lag." This idea was substantiated by the experiments, which show that by rebreathing a small volume the results are much lower than those obtained by breathing the mixtures, whereas in rebreath-

ing the large volume, in which time is allowed for the "lag," the results are somewhat the same, though in the higher concentrations there is still a variation, with the rebreathing giving the lower result.

This fact explains why the results obtained by Peabody (1915) rebreathing a small volume, are lower than those of Davies, Brow and Binger (1925) rebreathing a large volume, and may also offer an explanation as to why the results of the latter do not fit their ideal formula at the higher concentrations of carbon dioxide.

The experiments in which carbon dioxide determinations of the alveolar air have been done show clearly that the carbon dioxide tension of the arterial blood is slightly decreasing while the respiration is increasing. When the carbon dioxide mixture is replaced by air at the end of the experiment, the tension of carbon dioxide in the arterial blood appears to be increasing while the respiration is decreasing. Although further work with blood analyses is necessary, it would appear that the explanation of the "lag" must lie in the time required to saturate the tissues with carbon dioxide at the higher tension. The fact that the "lag" is longer with high than with low percentages of carbon dioxide appears to confirm this view, which is also in line with the ideas of Winterstein (1921) and Gesell (1925) that the carbon dioxide tension (or hydrogen ion concentration) of the respiratory center, and not of the arterial blood, is the true chemical stimulus of respiration.

SUMMARY AND CONCLUSIONS

1. When air containing an increased amount of carbon dioxide is breathed, the maximum increase in respiration occurs only after the mixture has been breathed for some time. The length of this "lag" varies as the concentration of carbon dioxide inspired.

2. The reaction of any one subject to the same concentration of carbon dioxide at different times is in general constant, while the reactions of different individuals vary greatly.

3. Rebreathing experiments done with a small volume of air give too low a response for high concentrations of carbon dioxide, but rebreathing experiments with a large volume of air give results which compare favorably with those obtained by breathing mixtures of carbon dioxide and air.

4. When inspiring carbon dioxide mixtures, there is no delay in raising the carbon dioxide tension of the arterial blood. From this it appears that the "lag" in respiratory response may be explained by the time required to saturate the tissues (especially the respiratory center) to the new carbon dioxide tension.

I am indebted to Prof. E. K. Marshall, Jr., who made the original observations which suggested these experiments, and without whose untiring aid and many suggestions they would never have been completed; to my friends and colleagues who so kindly offered themselves as subjects for the experiments; and to Mr. R. B. Swope and the Southern Oxygen Company for furnishing us with the cylinders of compressed air which made the application of the method possible.

BIBLIOGRAPHY

Bock, A. V. and H. Field, Jr. 1924–25. Journ. Biol. Chem., lxii, 269.
Davies, H. W., G. R. Brow and C. A. L. Binger. 1925. Journ. Exper. Med., xli, 37.
de Almeida, M. O. 1917–18. Journ. de Physiol. et Pathol. Gen., xvii, 590.
1923. Ibid., xxi, 304, 445, 466.
Douglas, C. G. and J. S. Haldane. 1909. Journ. Physiol., xxxviii, 420.
Gesell, R. 1925. Physiol. Reviews, v, 551.
Hough, T. 1910. This Journal, xxvi, 156.
1911. Ibid., xxviii, 369.
Lindhard, J. 1911. Journ. Physiol., xlii, 337.
Peabody, F. W. 1915. Arch. Int. Med., xvi, 846.
Scott, R. W. 1920. Arch. Int. Med., xxvi, 544.
Winterstein, H. 1921. Pflüger's Arch., clxxxviii, 293.

PHOSPHORIC ACID CHANGES IN WORKED MAMMALIAN MUSCLE

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Received for publication September 24, 1927

In the total phosphorus of muscle may be recognized quite distinctly the fraction soluble in acid extractives and precipitated by reagents specific for inorganic phosphates. As recent work points to a relation between intermediary carbohydrate metabolism and the phosphates of muscle it seems that studies of the phosphates should be controlled by precautions similar to those found essential for investigating lactic acid changes. Consequently the experiments described were performed with surviving decapitated cats, in which the muscles were previously carefully dissected except for nervous and circulatory connections. Upon the completion of the experimental period, the muscles were removed and immersed in liquid air. In this way there was no interruption or delay between the end of the experiment and fixation of the muscle, and disturbances from prolonged anesthesia were avoided. That the alteration incidental to removal of muscles so prepared is uniformly slight appears from the small lactic acid content of the controls.

The fact that surviving muscles retain an intact circulation makes difficult the evaluation of changes whose products suffer prompt circulatory removal. The other extreme is furnished by muscles either excised or *in situ* in dead animals, where the advantage of retention of all products is offset by the dead or dying condition of the muscle. A certain series of experiments was likewise performed with muscles frankly no longer in normal state. From these data are presented observations on changes in the acid soluble inorganic phosphates and their relation to lactic acid and glycogen.

The most extensive evidence on a relation of phosphates to the carbohydrate metabolism of muscle follows from the studies of Embden and his collaborators on lactacidogen (Embden, Griesbach and Schmitz, 1914). Starting from the observation that phosphoric acid as well as lactic acid increased in pressed muscle juice, a phosphate-carbohydrate was suggested as the precursor of lactic acid and named lactacidogen. Subsequent work in Embden's laboratory supports the view of this relation, culminating perhaps in the separation of hexose phosphates from muscle (Embden and

Zimmermann, 1924). The most recent results (Embden, 1927) identify the lactacidogen from fresh rabbit muscle as a hexose monophosphate.

This conception of the situation of hexose phosphates in the intermediary metabolism of carbohydrates in muscle follows from the scheme presented for alcoholic fermentation.

In formulating the reactions involved in the production of muscle energy Meyerhof (1924) has incorporated a similar series of changes whereby the breakdown of glycogen to lactic acid proceeds through the intermediary of a hexose phosphate. The hypothetical reactions agree in energy values with the rather approximate physiological data available. In general, the intermediary position of a carbohydrate phosphate such as Embden's lactacidogen in muscle metabolism corresponds with the observations.

The experiments of Best, Hoet and Marks (1926) showed a plain decrease in blood phosphate during insulin-sugar perfusion of eviscerated spinal cats. But additional amounts of perfused phosphate did not cause an increase of lactacidogen (Best and Marks, 1926).

There are numerous contradictions in the evidence on the effect of work upon the acid soluble inorganic phosphorus of muscle. In accordance with its hypothetical position and the considerably increased phosphorus of the blood and in the urinary excretion after exercise (Havard and Reay, 1926), an increased concentration in muscle might be expected. This has been demonstrated by Embden, Lehnarts and Hentschel (1927) in excised frog muscle, with corresponding lactacidogen reduction. Andrews (1925) reported that after short periods of stimulation dog or cat muscle showed a diminished inorganic phosphate and lactacidogen content. After several hours' stimulation of decerebrate cat muscles or in dying animals after a shorter time Cuthbertson (1925) observed increased phosphate and diminished lactacidogen. The discordant evidence is sufficiently extensive to suggest that some factors have not been controlled or considered in the usual experiments, so that we cannot offhand estimate the significance of the results.

Methods. The spinal cat preparations were made with cats quickly anesthetized with chloroform followed by ether. Within a half hour after initial anesthesia decapitation was complete and respiration maintained with a Brodie pump. A subsequent period of from half an hour to an hour followed during which the muscles were exposed and dissected, retaining circulatory and nervous connections intact, but freed from surrounding tissue except for the muscle insertions. About a half hour was then allowed for recovery of the muscles from stimuli received during preparation, the whole period serving also for the effects of anesthesia to wear off. In some experiments final removal of muscle did not occur until six hours after decapitation, during which time circulation remained good. The animals were kept warm with an electric hot pad.

Fixation of muscles was secured by immersion in liquid air. Grinding in a mortar made possible representative sampling from the well mixed muscle powder.

Determinations of acid-soluble inorganic phosphorus alone were made on the 5 per cent trichloracetic acid filtrate after three hours' extraction. Where lactic acid was also determined in the same sample aliquots were taken from the Schenck precipitation filtrate.

Phosphorus so determined represents the acid soluble inorganic fraction. This fraction, termed "A" for convenience, has been tacitly assumed to show the normal concentration in living muscle but the recent work of Eggleton and Eggleton (1927) and of Fiske and Subbarrow (1927) shows that the true natural inorganic phosphorus is probably only about twenty per cent of this amount. Their methods indicate another combination than that of carbohydrates with phosphorus, so that until new evidence requires modification of the view, we may assume that the conventionally determined acid soluble inorganic phosphorus represents a definite entity of physiological significance.

The second phosphorus fraction determined is that found after two hours' incubation of muscle powder in two per cent sodium bicarbonate at 40 degrees. This quantity, termed "B," represents likewise, according to Embden, Schmitz and Meincke (1921) a definite physiological entity, the maximum hydrolyzable phosphorus. The part so hydrolyzed is considered by Embden to have originated by enzymatic splitting of hexose phosphate esters or lactacidogen. Consequently the lactacidogen may be determined by the difference between the "A" and "B" phosphorus fractions and is conventionally expressed in terms of this difference.

Inorganic phosphorus determinations were at first made colorimetrically according to Briggs' modification of the Bell-Doisy method,—later by the more reliable gravimetric method of Embden (1921). Results from either Schenck or trichloracetic acid extraction agreed.

The "A" lactic acid was determined in the Schenck filtrate according to the Hirsch-Kauffmann (1924) description of Clausen's method. A second ("B") lactic acid determination was made upon a Schenck filtrate after 2 hours' incubation of muscle powder in two per cent disodium phosphate at 40 degrees, which condition gives, according to Meyerhof, maximal lactic acid production.

Glycogen determinations were made according to Pflüger's method, the glycogen being determined and expressed as glucose by either the Bertrand or Shaffer-Hartmann (1920) method.

The first series of experiments was arranged to secure by means of continued tetanic stimulation the maximal amount of contraction in a short period, on the hypothesis that this procedure would most likely cause accumulation of the effects of fatigue more rapidly than the blood supply

would bring recovery. Gracilis and semitendinosus muscles in each leg of decapitated cats were dissected and one side was subjected to direct tetanic stimuli producing maximal contraction, until appreciable response failed. At this time the control and stimulated muscles were removed, frozen in liquid air and samples determined for "A" and "B" phosphorus, dry substance and glycogen. The results are recorded in table 1.

The figures indicate in three cases increased "A" phosphate and in two decreased, with the lactacidogen increased in two muscles, decreased in one and unchanged in one. Apparently the experimental conditions of

TABLE 1

Effect of tetanic stimulation to fatigue upon the phosphorus and glycogen

Surviving decapitated cat muscles

NUMBER	MUSCLE	TIME	DRY SUBSTANCE	P			GLYCOGEN
				A	B	B−A	
		minutes	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4	l. gracilis	0		0.096	0.120	0.024	
	r. gracilis	15		0.103	0.161	0.058	
5	r. gracilis	0	24.45	0.118	0.143	0.025	0.87
	l. gracilis	20	20.06	0.089	0.134	0.045	0.23
				(0.106)	(0.164)	(0.058)	(0.28)
	r. semitend.	0		0.091	0.129	0.038	0.81
	l. semitend.	15		0.103			0.50
6	r. gracilis	0	25.65	0.085	0.135	0.050	0.28
	l. gracilis	30	22.41	0.088	0.132	0.044	0.065
				(0.101)	(0.151)	0.050	(0.074)
	r. semitend.	0	24.98	0.087	0.143	0.056	0.31
	l. semitend.	15	22.71	0.077	0.132	0.055	0.087
				(0.85)	(0.145)	(0.060)	(0.096)

Note: Parentheses indicate recalculation on basis of same per cent dry substance as control.

P determined—Briggs. Glycogen as glucose—Bertrand.

rapid tetanic stimulation to fatigue affect the phosphate situation, but not uniformly.

Certain concomitant changes agree to show that the muscles are otherwise uniformly altered by this procedure. The glycogen is notably diminished in the worked muscles, but without apparent relation to the phosphate changes. The change in water content of the muscles shows a striking increase. But even recalculation of phosphorus for the dilution occurring during contraction does not make a more consistent picture.

In the second series of experiments (reported in table 2) one muscle in each pair used was stimulated through the nerve and then directly to a long continued series of maximal contractions, but not to complete fatigue.

TABLE 2

Effect of protracted interrupted stimulation on phosphorus, lactic acid and glycogen

Surviving decapitated cat muscle

EXPERIMENT NUMBER	MUSCLE	TIME	DRY SUBSTANCE	P			LACTIC ACID			GLYCOGEN
				A	B	B-A	A	B	B-A	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
VIII 9	r. gastrocn.	0	24.7	0.089	0.170	0.081	0.035	0.70	0.66	0.41
	l. gastrocn.	1 hr. 15 m.	22.8	0.101	0.138	0.037	0.23	0.36	0.13	0.13
		at 50/m.		(0.109)	(0.149)	(0.040)	(0.25)	(0.39)	(0.14)	(0.14)
		20 m.								
		at 80/m.								
		Not exhausted								
VIII 10	l. gracilis	0	23.38	0.104	0.151	0.047	0.029	0.70	0.67	0.59
	r. gracilis	50 m.	20.49	0.101	0.141	0.040	0.097	0.43	0.33	0.26
		Tetanus		(0.115)	(0.161)	(0.046)	(0.11)	(0.49)	(0.38)	(0.30)
		Not fatigued								
	l. gastrocn.	0	24.57	0.097	0.148	0.051	0.008	1.03	1.02	0.90
	r. gastrocn.	1 hr. 40 m.	23.79	0.111	0.151	0.040	0.35	0.62	0.27	0.46
		at 50/m.		(0.115)	(0.156)	(0.041)	(0.36)	(0.64)	(0.28)	(0.48)
		Not completely exhausted								
VIII 11	r. gastrocn.	0	23.6	0.110	0.175	0.065	0.006	0.35	0.34	0.60
	l. gastrocn.	2 hrs.	22.8	0.114	0.167	0.053	0.073	0.25	0.18	0.22
		at 25/m.		(0.118)	(0.173)	(0.055)	(0.076)	(0.26)	(0.18)	(0.23)
		1 hr. 22 m.								
		at 50/m.								
		Not fatigued—worked against tension, stimulated through nerve								

Note: Parentheses indicate recalculation to same per cent dry substance as control.

If change in water content is significantly related to the amount of contractile energy expended, then protracted interrupted stimulation is not so exhausting as tetanic. As the muscles were not completely fatigued and the degree of glycogen depletion was not great, the worked muscles appear by these criteria to have suffered less than from the shorter tetanic stimulation.

The lactacidogen, judged by the difference between "A" and "B" phosphorus, is plainly diminished in each experiment, including the gracilis subjected to 50 minutes' tetanus. Experiment VIII, 9 shows in the worked muscle the greatest lactacidogen decrease, the least available ("B"–"A") lactic acid, and the greatest increase in water content of the muscles receiving interrupted stimuli. Although the lactacidogen difference between the worked gastrocnemii of VIII, 11 and VIII, 10 is small, VIII, 11 shows a greater lactacidogen decrease, less "B" lactic acid and equal water increase. The tetanized gracilis of VIII, 10 does not appear strictly comparable with the gastrocnemii, for it shows the greatest lactacidogen, available glycogen, and lactic acid supplies after stimulation, but also a greater increase in water.

Arranging the worked muscles in order of amount of available lactic acid ("B"–"A") beginning with the least, makes the series VIII, 9, VIII, 11, VIII, 10 gastrocnemius and VIII, 10 gracilis. The same order holds for amount of lactacidogen decrease, although the differences are not large.

No significance appears in the amounts of initial ("A") lactic acid. But in the worked muscles the available lactic acid is in three cases out of four approximately equal to the glycogen supply; while in the resting muscles, except one, "B"–"A" lactic acid exceeds glycogen.

The indications from this series of experiments show plain increase in "A" inorganic phosphorus and decrease in lactacidogen. The evidence is suggestive that lactacidogen utilization exceeds its resynthesis when a certain degree of fatigue is reached. The degree of fatigue necessary is apparently not measured by the rough glycogen depletion, for in series 1, where glycogen depletion was even greater after short tetanus, there was no uniform lactacidogen change.

The conditions of series 2 compare with those under which Cuthbertson (1925) found the lactacidogen diminished. Those in series 1 are comparable to the conditions where Andrews also (1925) found a lactacidogen reduction. Andrews' experiments showed uniformly a diminished "A" phosphorus, while series 1 shows the "A" change variable in sign under the conditions of short tetanus.

The third series of experiments (table 3) proposed to modify the circulation to one stimulated muscle. The two muscles were directly tetanized simultaneously while the circulation to one was impeded but not shut off. This was accomplished by drawing upon a loose ligature around

the principal vessels. That a certain amount of circulatory restriction occurred shows from the similar differences in the restricted muscles.

The effect of circulatory restriction appears in diminished glycogen supply and water content. The two restricted gracilis muscles utilized more glycogen than the single gastrocnemius, but had a scarcely perceptible lactacidogen reduction, although the latter is more conspicuous in the gastrocnemius. There is generally more lactacidogen in the gastrocnemii than in the graciles in each series and it may be more sensitive to influences provoking its utilization.

TABLE 3

Effect of restriction of circulation during tetanic stimulation upon phosphorus and glycogen

Surviving decapitated cat muscles

NUMBER	MUSCLE	TIME	DRY SUBSTANCE	P			GLYCOGEN
				A	B	B–A	
		minutes	*per cent*	*per cent*	*per cent*	*per cent*	
7	l. gracilis	15	24.08	0.103	0.143	0.040	0.41
				(0.108)	(0.150)	(0.042)	(0.43)
	r. gracilis	15+	25.31	0.106	0.145	0.039	0.34 artery and vein constricted
8	l. gastrocn.	9	22.81	0.105	0.152	0.047	0.30
				(0.107)	(0.155)	(0.048)	(0.31)
	r. gastrocn.	9+	23.33	0.104	0.140	0.036	0.26 artery constricted
	l. gracilis	15+	24.04	0.125	0.160	0.035	0.22
				(0.131)	(0.168)	(0.037)	(0.23)
	r. gracilis	15	25.20	0.130	0.163	0.033	0.14 artery and vein constricted

Note: Parentheses indicate recalculation on basis of same per cent dry substance as control.

P—according to Embden—gravimetric. Glycogen as glucose—(7) Bertrand, (8) Shaffer-Hartmann.

These experiments imply again that maintenance of lactacidogen is a function affected along with glycogen supply and water content by the circulation. The significance of the small lactacidogen reduction gains in importance because of its agreement with the other two changes, which consistently attend the processes of muscle contraction.

The fourth series of experiments (reported in table 4) shows plainly the increased inorganic phosphate ("A") after anaerobic work, but the "B" phosphate is also increased, so that no consistent reduction in lactacidogen appears. There is likewise an increase in "B" lactic acid of the worked muscle, but the total amount produced there is less than the available glycogen might supply. A similar discrepancy appears in the worked muscle, whose new lactic acid (B–A) is less than the glycogen available.

The degree of glycogen depletion in the worked muscles is small, and the lactic acid produced is much less than the glycogen utilized. These experiments may be comparable to those on impedance of the circulation in that other conditions produce fatigue so quickly as to preclude perceptible changes in the lactacidogen situation.

The fifth series of experiments (reported in table 5) takes postmortal changes of muscles left *in situ* for various periods up to the development of rigor. "A" lactic acid was not determined in the gracilis and semitendinosus controls for economy of material, experience having shown that

TABLE 4

Effect of fatigue under anaerobic conditions on phosphorus, lactic acid and glycogen

Muscle excised from surviving decapitated cat

EXPERIMENT NUMBER	MUSCLE	TIME	P			LACTIC ACID			GLYCOGEN
			A	B	B–A	A	B	B–A	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
VIII 9	r. gracilis	0	0.111	0.130	0.019		0.413		0.22
	Rested in N_2								
	l. gracilis	18 50/m.	0.123						0.20
	Fatigued in N_2								
VIII 12	gracilis	0	0.108	0.143	0.035	0.024	0.439	0.42	1.08
	gracilis	52 m. 60/m.	0.135	0.166	0.031	0.31	0.53	0.22	0.50
	Fatigued in H_2								
	gastrocn.	0	0.116	0.151	0.035	0.009	0.53	0.52	0.92
	gastrocn.	16 m. 50/m.	0.124	0.162	0.038	0.29	0.62	0.33	0.45
	Fatigued in H_2								

Note: Parentheses indicate recalculation to same per cent dry substance as control.

the amount of lactic acid in a resting muscle is typically small enough to be practically disregarded.

The "A" phosphorus after 45 minutes increases with time while "B" remains constant, giving a corresponding lactacidogen (B—A) decrease. The "A" lactic acid progressively increases in about the same amount as glycogen decreases, again excepting the first 45 minute experiment.

The gracilis after 45 minutes shows figures similar to the results of series 4, where the time for postmortal changes is likewise short. The "lactacidogen" value is even increased and the available glycogen equals about

twice the lactic acid produced. These facts in both experiments suggest that the anomalies with the hypothetical position of lactacidogen originate in failure to consider the functional character of such an intermediary. In an active system it should definitely serve to influence the dissimilation of glycogen either by opposition of its supposed function in synthesis or by facilitating the accumulation of other glycogen decomposition products than lactic acid. The same causes may affect the other experiments with still living muscle to prevent the quantitative conversion of glycogen to lactic acid, but they cannot be evaluated with an intact circulation.

Apparently the semitendinosus and gastrocnemius have proceeded with postmortal changes and so are quite different from the gracilis, which is comparable with normal muscles. The lactacidogen is significantly reduced, although the glycogen is by no means expended. And, although our interest may magnify the significance, it is conspicuous that the lactic

TABLE 5

Postmortal changes in phosphorus, lactic acid and glycogen in situ

Surviving decapitated cat muscle

EXPERIMENT NUMBER	MUSCLE	TIME	P			LACTIC ACID			GLYCOGEN
			A	B	B−A	A	B	B−A	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
IX 2	l. gracilis	0	0.127	0.152	0.025		0.30	0.30	0.50
	r. gracilis	45 m.	0.125	0.155	0.030	0.145	0.38	0.23	0.46
	l. semitend.	0	0.128	0.166	0.038				
	r. semitend.	2½ hrs.	0.157	0.162	0.005	0.22	0.49	0.27	0.25
	l. gastrocn.	0	0.111	0.178	0.067	0.07	0.50	0.43	0.52
	r. gastrocn.	6	0.146	0.178	0.022	0.38	0.54	0.16	0.14

acid produced during hydrolysis ("B"–"A") balances well with the available glycogen.

This agreement between available lactic acid and glycogen fails to appear in normal resting muscles of the controls for series 2, 4 and 5 and likewise in the less worked muscles of series 4, and the gracilis 45 minutes after death. All of these muscles have unimpaired lactacidogen supplies. On the contrary, in series 2 and 4 we find that with more distinct reduction in the amount of lactacidogen comes a closer agreement between the amount of glycogen available and the ("B"–"A") lactic acid (produced by incubation of muscle at 40° in 2 per cent Na_2HPO_4).

The figures in table 6 show the amount of lactacidogen decrease, "B"–"A" lactic acid and glycogen. These figures show that the agreement between "B"–"A" lactic acid and glycogen is excellent where the lactacid-

ogen reduction is distinct. In normal muscles there is no sign of agreement.

Apparently then, a displacement of the acid soluble inorganic phosphorus relations in the muscle is connected with the utilization of glycogen. The evidence does not indicate whether a store of products other than lactic acid ordinarily accumulates from glycogen decomposition, or whether the accumulation might not be in the direction toward glycogen

TABLE 6

Comparison of lactacidogen depletion with available lactic acid and glycogen

EXPERIMENT NUMBER	LACTACIDOGEN DECREASE	B−A LACTIC ACID	GLYCOGEN	("B"−"A")-GLYCOGEN	
				Fatigued	Normals
IX 2. Gastrocnemius	0.045	0.16	0.14	+0.2	−0.9
		(0.43)	(0.52)		
VIII 9. Gastrocnemius	0.044	0.13	0.13	0.0	+0.25
		(0.66)	(0.41)		
IX 2. Semitendinosus	0.033	0.27	0.25	+0.2	
VIII 11. Gastrocnemius	0.012	0.18	0.22	−0.4	−0.26
		(0.34)	(0.60)		
VIII 10. Gastrocnemius	0.011	0.27	0.46	−0.19	+0.12
		(1.02)	(0.90)		
VIII 10. Gracilis	0.007	0.33	0.26	+0.7	+0.8
		(0.67)	(0.59)		
		(0.30)	0.50		−0.20
		(0.23)	0.46		−0.23
Average difference				0.6	0.18

Note: Parentheses indicate corresponding muscles with normal lactacidogen.

synthesis from lactic acid. The number of possible alternatives is too great for the present evidence to justify their consideration, but an encouraging opening for further investigation is presented.

CONCLUSIONS

Tetanic stimulation to fatigue may either increase or decrease the "A" phosphorus and likewise the "B" fraction. As a result the effects on lactacidogen ("B"–"A") are variable, the lactacidogen increases being more conspicuous.

Prolonged interrupted stimulation increases the "A" phosphorus and

usually diminishes the "B" fraction, with consequent decrease in lactacidogen. Restriction of the circulation to a working muscle decreases the lactacidogen slightly, but a short period of anaerobically performed work does not have this effect. Only a considerable period after death does the lactacidogen decrease become conspicuous.

The water content of stimulated muscles increases rapidly, most conspicuously in tetanized muscles. Glycogen disappearance and the increase of lactic acid are also plain in the worked muscles. The two latter changes also characterize postmortal changes in muscle, and all three effects of muscle contraction are emphasized in working muscles with obstructed circulation.

Among normal muscles it is characteristic that the lactic acid available on hydrolysis with 2 per cent sodium diphosphate is not equivalent to the available glycogen. Among the muscles showing depletion of lactacidogen, either following work or death, the lactic acid produced and glycogen are about equivalent. This evidence points to a distinct change in the results of intermediary carbohydrate metabolism when the normal phosphorus relations are displaced.

Note: I wish to thank Dr. J. J. R. Macleod for suggestions that prompted the work and for help and advice during the early experiments.

Thanks are also due to Mr. P. H. Wells for reliable and competent assistance.

BIBLIOGRAPHY

ANDREWS, S. 1925. Biochem. Journ., xix, 242.

BEST, C. H., J. P. HOET AND H. P. MARKS. 1926. Proc. Roy. Soc. (London) B c, 32.

BEST, C. H. AND H. P. MARKS. 1926. Proc. Roy. Soc. (London) B c.

CUTHBERTSON, D. P. 1925. Biochem. Journ., xix, 896.

EGGLETON, P. AND G. P. EGGLETON. 1927. Biochem. Journ., xxi, 190.

EMBDEN, G. 1921. Zeitschr. f. physiol. Chem., cxiii, 138.
1927. Klin. Wochenschr., 6 Jahrg., Nr. 14.

EMBDEN, G., W. GRIESBACH AND E. SCHMITZ. 1914. Zeitschr. f. physiol. Chem., xciii, 1.

EMBDEN, G., E. LEHNARTS AND H. HENTSCHEL. 1927. Zeitschr. f. physiol. Chem., clxv, 255.

EMBDEN, G., E. SCHMITZ AND P. MEINCKE. 1921. Zeitschr. f. physiol. Chem., cxiii, 10.

EMBDEN, G. AND M. ZIMMERMANN. 1924. Zeitschr. f. physiol. Chem., cxli, 225.

FISKE, C. H. AND Y. SUBBARROW. 1927. Science, lxv, 401.

HAVARD AND REAY. 1926. Journ. Physiol., lxi, 35.

HIRSCH-KAUFFMANN. 1924. Zeitschr. f. physiol. Chem., cxl, 25.

MEYERHOF, O. 1924. Naturwissenschaften, 182.

SHAFFER-HARTMANN. 1920. Journ. Biol. Chem., xlv, 365.

THE RELATIONSHIP OF GLYCOGEN FORMATION IN THE MUSCLES TO THE PANCREAS AND TO EPINEPHRIN

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Received for publication September 24, 1927

The recent researches concerning the chemical processes underlying muscular contraction have given to the glycogen of muscles a much greater prominence than it had previously been suspected to possess, and that it must be regarded as fulfilling a function which is essentially different from that of the glycogen of the liver has been made evident by studies on its behavior in various experimental and pathological conditions, among which may be mentioned the effects of hepatectomy, of insulin and of diabetes. The glycogen of the liver is to be considered partly as an intermediary substance in the new formation of glucose out of protein and fat, and partly as a storage form of excess carbohydrate, whereas the glycogen of the muscles has but one function, namely, to serve as the mother substance of lactic acid formation. This implies that in conditions of muscular inactivity no quantitative change should occur in the glycogen of the muscles, and that such indeed is the case has been shown by Best, Hoet and Marks (1926) who found no difference in the percentage amounts of glycogen in corresponding muscles of the two legs in decapitate eviscerated cats after intervals of several hours. During the intervals what energy the muscles did evolve must have been derived either from other substances than glycogen, or from glycogen which was re-formed out of blood sugar at exactly the same rate as it was being converted into lactic acid. These workers also found that when excess of insulin is injected into the preparations more glycogen becomes formed in the muscles. These interesting observations formed the starting point of the present investigation, in which, besides confirming their results, it has also been shown that glycogen formation in the muscles depends on the presence of the pancreas and is uninfluenced by epinephrin.

EXPERIMENTS WITH INSULIN. *Methods.* Cats from which food had been withheld for 24 hours were used immediately after decapitation under ether, and the establishment of artificial respiration. Ligatures were applied successively to the mesenteric and coeliac arteries, the lower end of the rectum, the esophagus and the portal vein. The stomach and intestines, spleen and pancreas were then removed. To obtain the muscles

without injury, the sartorius, gracilis and gastrocnemius were dissected free in both legs, care being taken not to injure their blood vessels. These muscles were subsequently removed by cutting across their insertions, and as each was removed it was immediately dropped into hot KOH. An interval of at least 15 minutes always elapsed between the unsheathing of the muscles and their removal. The muscles of the left leg were used as controls and the muscles of the right leg finally removed. This procedure is essentially the same as that of Best, Hoet and Marks. A 10 per cent glucose solution was then injected over a period of two and one-half hours

TABLE 1

Per cent glycogen

	LEFT	RIGHT	DIFFERENCE
1. Sartorius	0.590	0.596	0.006
Gracilis	0.610	0.625	0.015
Gastrocnemius	0.711	0.763	0.052
Average	0.633	0.661	0.028
2. Sartorius	0.760	0.704	0.056
Gracilis	0.854	0.845	0.009
Gastrocnemius	0.799	0.777	0.022
Average	0.804	0.775	0.029

TABLE 2

Per cent glycogen

	LEFT	RIGHT	DIFFERENCE
Experiment 3	0.720	0.708	0.012
Experiment 4	0.707	0.692	0.015
Experiment 5	0.619	0.692	0.011
Experiment 6	0.630	0.610	0.020

into the jugular vein by means of continuous injection (Woodyat) apparatus.

The blood sugar was determined by the Shaffer-Hartmann method, and glycogen by Pflüger's method, the sugar formed by the acid hydrolysis of the glycogen being estimated by Shaffer-Hartmann method. By careful neutralization of the hydrolysed solution and by strict observance of standard conditions it was found, by numerous controls, that this method is accurate and dependable.

Results. In a series of control experiments it was found in confirmation of others that the glycogen content in different muscles of the same animal, and in similar muscles of different animals, varies greatly, but that the

glycogen content of the corresponding muscles of the two legs of the same animal agree fairly closely with each other. The results of two typical experiments are given.

A 10 per cent glucose solution was injected through the jugular vein at the rate of 14 cc. (1.4 grams) per hour, and the right leg muscles were removed after 2 hours and 30 minutes' injection (3.5 grams glucose). These experiments are numbered 3, 4, 5 and 6, and the results are shown in table 2. In no. 3 the percentage of blood sugar rose from 0.243 to 0.513; in experiment 4, from 0.235 to 0.537; in experiment 5, from 0.217 to 0.423, and in experiment 6, from 0.127 to 0.375. The glycogen content was practically unaffected by the injection.

In all four cases the right leg shows slightly less glycogen than the left, but the maximum difference of 0.02 per cent warrants no discussion in view of the fact that the control experiments (see table 1) showed differences of the same magnitude. These experiments support the findings of Best, Hoet, Marks, and of Hatcher and others, that the muscle glycogen of an eviscerated cat is not increased by perfusion with blood containing excess

TABLE 3

Per cent glycogen

	LEFT	RIGHT	DIFFERENCE
Experiment 7	0.408	0.473	0.075
Experiment 8	0.631	0.656	0.025
Experiment 9	0.509	0.582	0.073

of glucose. On the other hand, as the next group of experiments show, when the abdominal viscera are not removed, the other conditions of the experiment being the same, glycogen is formed.

Effect of glucose injection without evisceration. In experiments 7, 8 and 9 the abdominal viscera were not removed and a 10 per cent glucose solution was injected intravenously at the rate of 14 cc. (1.4 grams) per hour for 150 minutes continuously (a total of 3.5 grams glucose).

Experiment 7. Cat, 4.1 kgm. The initial blood sugar was 0.223, after injection it rose to 0.301 per cent.

Experiment 8. Cat, 2.15 kgm. The initial blood sugar was 0.191 per cent; after injection it rose to 0.306 per cent.

Experiment 9. Cat, 3.2 kgm. The initial blood sugar was 0.231 per cent; after injection it rose to 0.285 per cent. The average glycogen content of the muscles in these experiments is given in table 3.

The results show clearly that glycogen is formed in the muscles when excess of sugar is present in the blood with the abdominal viscera intact. Since under these conditions the internal secretion of insulin from the

pancreas would still be going on the next step was to see whether glycogen formation would occur after removal of the pancreas only.

The effect of injection of glucose in depancreatised cats. Pancreatectomy was performed 24 hours previous to decapitation, in experiments 10 and 11 and immediately preceding it in experiment 12. The total amount of glucose injected over an interval of 170 minutes in each case was 35 cc. of 10 per cent solution (3.5 gm.).

Experiment 10. Cat, 3.1 kgm. Depancreatised 24 hours previous to decapitation. The initial blood sugar was 0.186 per cent; after injection it rose to 0.330 per cent.

Experiment 11. Cat, 3.7 kgm. Depancreatised 24 hours previous to decapitation. The initial blood sugar was 0.162 per cent; after injection it rose to 0.309 per cent.

Experiment 12. Cat, 4.60 kgm. Depancreatised immediately after decapitation. The initial blood sugar was 0.169 per cent; after injection it rose to 0.313 per cent. The average muscle glycogen content is shown in table 4.

TABLE 4

Per cent glycogen (average of 3 muscles)

	LEFT	RIGHT	DIFFERENCE
Experiment 10	0.416	0.408	0.008
Experiment 11	0.459	0.443	0.016
Experiment 12	0.555	0.549	0.006

The results show that glycogen formation does not occur in the muscles after pancreatectomy. The results also indicate that the presence of the liver does not in itself determine whether or not glycogen formation in the muscles will occur; for in the depancreatised animals the circulation through this organ was undisturbed. It is of interest to note in this connection that in eviscerated preparations a certain amount of blood passes from the liver into the main circulation (Best, Hoet, Dale, Marks and others) without any glycogen formation occurring in the muscles.

A difference in the behaviour of the muscle glycogen can also be demonstrated in non-eviscerated cats as compared with cats depancreatised 24 hours previously, when after decapitation they are merely kept for 2½ hours on the warmed table, a small amount (3 to 5 cc.) of sugar-free Locke's solution being injected to maintain a good circulation. The results of such observations are shown in table 5. Although quite distinct, the extent to which glycogen formation occurs in the muscles of animals still retaining the pancreas is seen to be small when compared with the large increase found by Best, Hoet and Marks following the injection of excess of insulin. The following observations confirm this glycogenetic effect of excess of insulin.

The effect of injection of glucose and insulin in eviscerated cats. In this series of experiments glucose was injected for thirty minutes at the rate of 1.4 grams per hour, before the administration of insulin, in order to prevent the blood sugar from falling below normal level.

Ten units of insulin were administered intravenously twice, at an interval of 60 minutes.

Experiment 18. Cat, 3.25 kgm. Decapitated and eviscerated. The blood sugar was 0.141 per cent at the start; after 7 cc. (30 minutes) glucose injection, 0.235 per cent; after 120 minutes of insulin injection, 0.149 per cent.

Experiment 19. Blood sugar was 0.247 just before injection of insulin; at the end of "insulin hour" it fell to 0.167 per cent.

TABLE 5

Average per cent of glycogen in muscles of decapitated cats

TYPE AND NUMBER OF EXPERIMENT	BLOOD SUGAR		GLYCOGEN OF MUSCLE		DIFFERENCE
	Initial	Final	Initial (left side)	Final (right side)	
	per cent	*per cent*			
Abdomen intact, 13	0.243	0.123	0.533	0.569	0.036
Abdomen intact, 14	0.178	0.125	0.294	0.294	0.00
Abdomen intact, 15	0.174	0.087	0.345	0.392	0.047
Depancreatised, 16	0.318	0.268	0.284	0.283	0.001
Depancreatised, 17	0.210	0.193	0.212	0.181	0.031

TABLE 6

Per cent of glycogen

	LEFT (BEFORE INSULIN)	RIGHT (AFTER INSULIN)	DIFFERENCE
Experiment 18	0.601	0.705	0.094
Experiment 19	0.374	0.528	0.154
Experiment 20	0.458	0.579	0.121

Experiment 20. Blood sugar was 0.214 per cent just before injection of insulin, and at the end of "insulin hour" it fell to 0.150 per cent. The average glycogen increase for the three muscles in these experiments is shown in table 6.

The increase in muscle glycogen after injection of insulin is seen to be much more marked than in animals in which the only source of insulin is in the pancreas.

If we calculate the ratio between the amount of sugar which disappeared and the glycogen which was formed in the muscles, on the same basis as that used by Best, Hoet and Marks, about 40 per cent of the disappearing glucose can be accounted for as glycogen. Thus in experiment 18 total glucose injected was 3.5 grams; the blood sugar fell from 0.235 to 0.149 per cent, i.e., loss of 0.086. The calculated "active volume" of this cat would be 540 cc. There was, therefore, a disappearance of

$\frac{540 \times 0.086}{100} = 0.46$ sugar in addition to the dextrose infused. Therefore, the total sugar disappearance was 3.96 grams. The average glycogen content of this animal before injection of insulin was 0.601; after insulin, 0.705, i.e., increase of 0.104 per cent. Therefore, sugar found as muscle glycogen was $\frac{1625[1] \times 0.104}{100} = 1.69$ gram. Then the balance will be as follows:

Total glucose lost	3.96
Total glucose found as glycogen	1.69
Missing	2.27

Although the foregoing results show that under the conditions of the experiment, glycogen cannot be built up in the muscles in the absence of insulin, either derived from the pancreas of the animal or injected from without, they do not warrant the conclusion that insulin is essential to such glycogen formation, for it has been the experience in this and in other laboratories that depancreatised dogs which have been diabetic over long periods of time, still have glycogen in their muscles. Thus between the third and fifth days after removal of insulin from depancreatised dogs Chaikoff found that the quantities of glycogen which disappeared from the muscles was very small when compared with the total energy meanwhile expended by the animal. Best and Scott found that a substance capable of lowering the blood sugar level in normal rabbits could be extracted from the muscles and other tissues of depancreatised dogs and, assuming this to be insulin, they concluded that there is an extra pancreatic source for the secretion of this hormone. If this be the case then glycogen formation in the muscles of depancreatised animals is readily explained, but in view of the fact that withdrawal of insulin from depancreatised dogs previously kept non-diabetic by its administration, is very rapidly fatal, we cannot consider this blood sugar lowering substance to be insulin in the ordinary sense. It is possible that insulin as ordinarily prepared is not a simple substance, but is composed of two or more hormones having different physiological functions, for example, one acting primarily on the process of gluconeogenesis and the other, on that of glycogenesis in the muscles.

EXPERIMENTS WITH EPINEPHRIN. One of the striking effects of epinephrin in normal animals is an immediate discharge of such an excess of glucose into the blood that hyperglycemia and glycosuria follow. The sugar comes from the liver,[1] and Markowitz (2) has shown that repeated injection of epinephrin into starved strychnine-treated rabbits does not lead to hyperglycemia, unless some glycogen remains in the liver. Removal of the liver causes as rapid a disappearance of blood sugar in depancreatised dogs as in non-diabetic animals, as shown by Macleod and

[1] This figure is 50 per cent of the body weight and represents the weight of the muscles.

Pearce (3). Mann and Magath (4) have shown that total removal of the liver in dogs results in a fall in blood sugar, accompanied by symptoms of hypoglycemia, and that epinephrin fails to cause any rise in blood sugar in the liverless animal, a result which has been confirmed in this laboratory by Soskin (5). These findings indicate that the blood sugar of diabetic, as well as of normal animals, is maintained by the liver. In the light of these facts it seemed of importance to determine whether epinephrin would have any influence on the glycogen of the muscles. Epinephrin was injected intravenously at a slow continuous rate into decapitated cats, some of which were also eviscerated. Altogether 0.1 cc. of 1–1000 adrenalin chloride solution diluted with 35 cc. Locke's solution was injected into each animal. The muscles of the left side were removed before the injection and those of the right side after it. The average percentages of glycogen, along with changes in blood sugar are shown in table 7.

TABLE 7

Effect of epinephrin on muscle glycogen

TYPE AND NUMBER OF EXPERIMENT	BLOOD SUGAR		AVERAGE GLYCOGEN		DIFFERENCE
	Initial	Final	Initial (left side)	Final (right side)	
	per cent	*per cent*	*per cent*	*per cent*	
Eviscerated, 21	0.240	0.198	0.412	0.331	−0.087
Eviscerated, 22	0.100	0.067	0.174	0.152	−0.022
Eviscerated, 23	0.145	0.120	0.595	0.463	−0.132
Eviscerated, 24	0.145	0.133	0.435	0.413	−0.022
Not eviscerated, 25	0.198	0.271	0.432	0.429	−0.003
Not eviscerated, 26	0.133	0.152	0.303	0.293	−0.010
Not eviscerated, 27	0.150	0.186	0.555	0.536	−0.019

Generally speaking the decrease of muscle glycogen following epinephrin was more pronounced in eviscerated, as compared with non-eviscerated cats. This difference may be explained by the fact, that eviscerated animals have no power in manufacturing glycogen, unless insulin is injected from outside. It is also possible that sugar derived from the liver accounts for the difference. It is significant also that the blood sugar was raised only in those animals still retaining the viscera. This confirms the view that epinephrin acts on glycogenolysis in the liver, but not on this process in the muscles. The experiment was modified in that glucose and insulin were first of all injected for some time so as to cause glycogen formation in the muscles, then epinephrin was injected. The procedure was as follows:

Immediately after decapitation and evisceration the left sartorius and gracilis muscles *a*, were removed, 10 per cent glucose was injected intravenously at the rate

of 14 cc. per hour for 150 minutes during which time two injections of insulin of 10 units each were given at one hour intervals. After this period, the left gastrocnemius and right sartorius muscles, *b*, were removed and adrenalin injected three times intravenously a total of 0.1 cc. adrenalin per kilo of body weight being used. After the "adrenalin period" the right gracilis and gastrocnemius muscles, *c*, were removed. In this way the left sartorius and gracilis muscles represent a normal state, while the left gastrocnemius and right sartorius muscles show the effects of glucose and insulin, and finally, the right gracilis and gastrocnemius muscles show the effect of adrenalin after insulin. The results are shown in table 8.

Glycogen increased considerably in the right sartorius and left gastrocnemius muscles, which received glucose and insulin, while in the right gracilis and gastrocnemius muscles, which were treated with epinephrin, it decreased in one of the experiments, but showed only a doubtful decrease in the other.

In many of the experiments a definite decrease of muscle glycogen occurred after epinephrin. Although the injection was given very slowly

TABLE 8

Per cent glycogen in muscles after insulin and sugar and subsequently epinephrin

	LEFT	RIGHT	DIFFERENCE
28. Sartorius *a*	0.219	*b* 0.361	0.142
Gracilis *a*	0.270	*c* 0.288	0.018
Gastrocnemius *b*	0.315	*c* 0.318	0.003 no fibrillation
29. Sartorius	0.221	0.367	0.146
Gracilis	0.262	0.221	0.041
Gastrocnemius	0.344	0.282	0.062 fibrillation

and evenly, it was observed that the animal frequently developed a hypersensitive state, so that during the "epinephrin period" there was a constant muscular fibrillation all over the body, and it is altogether likely that this accounts for the decrease in glycogen. Thus, in experiments 21, 23 and 29 fibrillation was prominent and glycogen diminution was pronounced, whereas in experiments 22, 24 and 28 no fibrillation was observed, and glycogen remained practically unchanged.

The conclusion seems warranted that epinephrin has no direct effect on muscle glycogen metabolism, this contrasting with its marked influence on hepatic glycogenolysis.

The glycogen of muscle has, therefore, an entirely different physiological significance from that of the liver. In the breakdown process within the intact muscle glycogen is not converted into glucose, but into lactic acid and the only condition under which it can indirectly contribute sugar to the blood is when this lactic acid is carried to the liver and there synthesized into glycogen, from which glucose is readily derived. Besides

the results presented in this paper, there are numerous others which support this view, thus: 1, progressive hypoglycemia which follows hepatectomy and the inability of epinephrin or asphyxia to affect the blood sugar in this condition (Mann and Magath, Soskin etc.). 2. The accumulation of lactic acid in proportion to the disappearance of glycogen in muscle immediately after death, whereas under the same condition in the case of liver no lactic acid appears, but only sugar (Macleod and Simpson). 3. The persistence of glycogen in the muscles in depancreatised dogs long after its virtual disappearance from the liver.

SUMMARY

1. The percentage of glycogen in the leg muscles of eviscerated spinal cats is not affected by injecting glucose intravenously over a period of 2 to 3 hours.

2. It becomes increased when glucose is injected along with insulin.

3. It also increases when glucose is injected without insulin into spinal cats from which the viscera have not been removed.

4. The organ responsible for these differences is the pancreas, since injection of glucose into depancreatised spinal cats causes no glycogen formation in the muscles.

5. Epinephrin does not stimulate the breakdown of muscle glycogen, nor does it give rise to hyperglycemia in eviscerated cats.

It is a great pleasure to express my indebtedness to Prof. J. J. R. Macleod for directing this research.

BIBLIOGRAPHY

Best, C. H., J. P. Hoet and H. P. Marks. 1926B. Proc. Roy. Soc., c, 44.
Macleod, J. J. R. and R. G. Pearce. 1922. This Journal, xxxii, 171.
Macleod, J. J. R. and W. W. Simpson. In press.
Mann, F. C. and T. B. Magath. 1922. Arch. Int. Med., lxxiii, 171.
Markowitz, J. 1925. This Journal, lxxiv, 24.
Soskin, S. 1927. This Journal, lxxxi, 381.

CARDIAC INHIBITION

I. The Effect of Vagal Inhibition on the Lactic Acid Content of the Terrapin's Heart

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Received for publication September 24, 1927

In the Croonian Lecture of 1882, Gaskell (1882) proposed the following theory to explain the inhibitory action of the vagus nerve, "The vagus is a tropic nerve of both the muscular tissue and the motor ganglion, meaning thereby that it increases the activity of the various formative processes going on in both these kinds of tissue by virtue of this quality." In support of this theory Gaskell (1882) reports experiments in which the auricular and ventricular beats increase in amplitude after inhibition and later (1887) he showed that there was an electrical variation of the opposite sign to that caused by a contraction on stimulation of the vagus nerve.

Only a few experiments have been done on the metabolism of the inhibited heart to test this theory. Barcroft and Dixon (1907) have shown that the oxygen consumption and the carbon dioxide elimination are diminished in the partially inhibited cat's heart. Wolfsohn and Ketron (1909–10) have obtained similar results in the totally inhibited dog's heart.[1] This type of experiment has been used as evidence against Gaskell's theory, yet experiments of this type are not conclusive evidence that anabolism is not increased. During vagal inhibition the heart is doing less work. This in itself would diminish the oxygen intake and CO_2 output and would not indicate any specific increase or decrease in anabolism.

Although our ideas of anabolism in muscular tissue have radically changed since Gaskell's work, the facts presented for the theory still remain and with the recent advances in our knowledge of the chemical dynamics of muscular contraction we are in a position to investigate the possible change in the lactic acid content of the heart during inhibition. There are three effects possible during inhibition, glycogen may be broken down to lactic acid but the mechanism may be so changed that the lactic acid formed is no longer able to bring about a contraction, inhibition may cause

[1] Since this paper was sent to the publishers, an article by Bohnenkamp and Friedmann appeared in Pflüger's Archiv (ccxvii, 664) in which they found similar results in frogs' hearts inhibited by vagal stimulation.

an increased formation of the glycogen from lactic acid as Gaskell's anabolic theory would suggest or the site of the inhibitory process may be in or before the mechanism that brings about the initial breakdown of glycogen to lactic acid.

In order to determine which of the above possibilities does take place, a study of the lactic acid concentration of the inhibited terrapin's heart was undertaken in atmospheres of oxygen and nitrogen.

METHODS. Meyerhof's (1920) method of extraction of the lactic acid from the muscle was used and the determination of the lactic acid was made by the Riegel[2] (1927) modification of Clausen's (1922) method. To test the methods, pure zinc lactate was prepared by adding calculated amounts of zinc chloride to lactic acid and recrystallizing the zinc lactate five times. The final product was free of chlorides and gave 27.54 per cent ZnO, the theoretical for $Zn(C_3H_5O_3)3H_2O$ being 27.36 per cent. A N/100 solution

TABLE 1

Percentage recovery of zinc lactate

	ZINC LACTATE	0.01 IODINE USED	0.01 IODINE THEORY	PER CENT RECOVERY
	mgm.			
Jan. 22	1.91	2.144	2.568	83.54
24	1.791	1.952	2.408	81.10
26	1.138	1.111	1.530	72.63
25	0.879	1.065	1.182	90.08
26	0.528	0.669	0.710	94.17
27	0.284	0.334	0.381	87.54
Average				84.84

was made from the zinc lactate and amounts varying from 1.91 mgm. to 0.289 mgm. of the lactate were added to alcohol and extracted and determined by the above mentioned methods. The results are given in table 1. The average of six determinations gave a yield of 84.84 per cent. All the subsequent determinations were corrected by dividing by the factor 0.85.

"Resting" and fatigue maximum lactic acid. As preliminary experiments the "resting" amount of lactic acid was determined separately for the auricle and the ventricle. A terrapin was placed in a refrigerator and after one or two hours in the refrigerator, the terrapin was quickly opened, the auricle separated from the ventricle and the tissues weighed. They were then dropped into separate mortars containing ice-cold alcohol and the lactic acid extracted and determined. The values obtained in grams per cent (table 2) for the auricle are slightly higher than for the ventricle.

[2] Through the kindness of Doctor Wilson, Miss Riegel's method was obtained before publication.

After the separation of the ventricle, the auricle beats several times during the weighing of the tissue which may be the possible explanation of the slight increase in the lactic acid concentration.

For the determination of the fatigue maximum, the hearts were suspended in a chamber through which nitrogen was passed. The heart was attached to a muscle lever that carried a load of 10 grams. The beats were allowed to decrease in amplitude until they had practically ceased and then the heart was quickly removed from the chamber and dropped into the freezing mixture. The lactic acid concentrations obtained are given in table 3. The fatigue maximum of the auricle is practically the same as that of the ventricle. In a former paper (Gemmill, 1927) it was found that the heat produced per beat per gram of tension developed for the auricle agreed with that of the ventricle and the above figures would also indicate a close similarity in the metabolic activity of the auricles and the ventricle.

TABLE 2

"Resting" lactic acid concentration

EXPERIMENT	1	2	3	4	5	6	AVERAGE
Auricle	0.031	0.044	0.032	0.041	0.037	0.030	0.036
Ventricle	0.022	0.036	0.026	0.021	0.031	0.018	0.026

TABLE 3

Fatigue maximum lactic acid concentration

EXPERIMENT	1	2	3	4	AVERAGE
Auricle	0.144	0.114	0.145	0.143	0.137
Ventricle	0.152	0.133	0.138	0.142	0.141

The results obtained on the terrapin's heart are higher than those obtained by Katz and Long (1925) for the stimulation maximum of the mammalian muscle, they obtained an average of 0.072 gram per cent in twenty experiments with a maximum yield of 0.130 and a minimum of 0.042 per cent. Redfield and Medearis (1926) obtained slightly higher values on the exercised strips from the terrapin's ventricle, values ranging from 0.12 to 0.22 gram per cent with an average of 0.16 per cent for ten experiments with the muscle at maximum fatigue.

Vagal stimulation. The vagus nerve was isolated in the neck of the terrapin and dissected out with the heart. The preparation was then mounted in the muscle chamber. Nitrogen was passed through the chamber and after the beats had begun to decrease in amplitude, the vagus nerve was stimulated with sufficient current to obtain complete inhibition. After an interval of time that varied with the different experiments, the

muscle was quickly removed from the chamber and dropped into the freezing mixture and the lactic acid concentration determined. Similar experiments were done in an atmosphere of oxygen. Control experiments in nitrogen and oxygen were made in which the hearts were allowed to beat for corresponding periods of time without stimulation of the vagus nerve.

RESULTS. The results of the experiments are given in table 4. The average result for the lactic acid content for vagal inhibitions in nitrogen is 0.093, the control experiments giving a value of 0.094. With the heart-nerve preparation in oxygen the experiments in which the vagus was

TABLE 4

	DATE	ATMOSPHERE	PER CENT LACTIC ACID	TIME IN CHAMBER	DURATION OF STIMULUS
				seconds	*seconds*
Vagus stimulated	March 26, 1927	Nitrogen	0.087	700	58
	30	Nitrogen	0.080	430	58
	April 6	Nitrogen	0.092	540	45
	25	Nitrogen	0.115	994	667
Average			0.093		
Control experiments	April 7	Nitrogen	0.085	863	
	19	Nitrogen	0.113	404	
	25	Nitrogen	0.083	311	
Average			0.094		
Vagus stimulated	April 19	Oxygen	0.081	204	41
	27	Oxygen	0.091	517	835
	May 10	Oxygen	0.086	782	588
Average			0.086		
Control experiments	April 27	Oxygen	0.091	300	
	May 6	Oxygen	0.079	359	
	9	Oxygen	0.070	455	
Average			0.080		

stimulated gave a lactic acid concentration of 0.086 per cent and the control experiments 0.080 per cent. The values for the experiments in which the vagus was stimulated and the controls varied from the average but in no case was there a greater variation in the stimulation experiment than in the controls.

DISCUSSION OF RESULTS. When a motor nerve to a muscle is stimulated, glycogen breaks down to lactic acid during the period of contraction and during the recovery period a portion of the lactic acid is built back to glycogen, the energy for the recovery period being derived from oxidation of

a part of the lactic acid. What portion of this mechanism stops during the stimulation of the inhibitory nerve? If there was an increase in anabolism as Gaskell's theory suggests, there would have been an increase in the process of reformation of glycogen with a decrease in the lactic acid content. If a change in the muscular mechanism had taken place so lactic acid was formed but no longer effective, there would have been an increase in the lactic acid content. But as the values obtained during stimulation and in the control experiments are practically the same, the place of inhibition must be in or before the mechanism that normally brings about a breakdown of glycogen to lactic acid and inhibition has no effect on the anabolism of glycogen from lactic acid.

CONCLUSIONS

1. The average "resting" concentration of lactic acid in the terrapin's auricle is 0.036 gram per cent and in the ventricle is 0.026 gram per cent.

2. The average fatigue maximum of lactic acid in the terrapin's auricle is 0.137 gram per cent and in the ventricle is 0.141 gram per cent.

3. Stimulation of the vagus nerve does not affect the concentration of the lactic acid in the muscle.

4. The site of inhibition in the terrapin's heart may be placed either in or before the mechanism that breaks down glycogen into lactic acid.

BIBLIOGRAPHY

GASKELL, W. H. 1882. Phil. Trans. Roy. Soc. London, clxxiii, 993.
1887. Journ. Physiol., viii, 404.
BARCROFT, J. AND W. E. DIXON. 1907. Journ. Physiol., xxxv, 182.
WOLFSOHN, J. M. AND L. W. KETRON. 1909–10. This Journal, xxv, Proc. p. xxv.
MEYERHOF, O. 1920. Pflüger's Arch., clxxxii, 232.
REIGEL, C. 1927. Journ. Biol. Chem., lxxiv, 123.
CLAUSEN, S. W. 1922. Journ. Biol. Chem., lii, 263.
GEMMILL, C. L. 1927. This Journal, lxxx, 668.
KATZ, L. N. AND C. N. H. LONG. 1925. Proc. Roy. Soć. B., xcix, 8.
REDFIELD, A. C. AND D. N. MEDEARIS. 1926. This Journal, lxxvii, 662.

THE REGULATION OF RESPIRATION

XVII. Effects of Intravenous Injection of Sodium Cyanide on the Acidity of the Arterial and Venous Blood

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Received for publication September 29, 1927

In the preceding paper, Gesell and Hertzman (1927), on the acidity of the circulating arterial and venous blood we considered effects of the administration of gaseous mixtures low in oxygen. In this paper we describe the effects of intravenous injection of sodium cyanide. The effects of low oxygen were attributed in the main to impaired oxidations in the tissues, to impaired coördination of the dual function of hemoglobin, and to direct effects of low oxygen pressure on the acidity of the blood. Sodium cyanide exerts, in general, these same three primary effects but the mode of action is somewhat different (Gesell, 1925). On the administration of low oxygen the impaired oxidations result from lowered oxygen pressure of the circulating blood, both the arterial and venous blood are reduced considerably below normal. With the administration of sodium cyanide oxidations are impaired by a direct effect of cyanide on the oxidative mechanism of the cell; both the venous and arterial blood are oxidized above normal. With an impaired supply of oxygen the disturbance of the coördination of the dual function of hemoglobin is attributable to a more or less permanently reduced condition of the circulating hemoglobin. With the administration of sodium cyanide the disturbed coördination is attributable to a more or less permanently oxidized condition of the hemoglobin. And finally the direct effect of reduced oxidation of the hemoglobin by low alveolar oxygen on the acidity of the blood is alkaline, whereas the direct effect of a highly oxidized condition of the hemoglobin resulting from the administration of cyanide is in the acid direction. Bearing these points in mind a comparison of this and the preceding paper is facilitated.

Methods. The procedure of these experiments was similar to that of earlier experiments. The acidity of the circulating arterial and venous blood was continuously recorded with the manganese dioxide electrode and the results checked with the hydrogen and quinhydrone electrodes on discontinuous samples. In earlier animal experiments, in which the continuous method was developed (Gesell and Hertzman, 1926), a discrepancy

between acid changes as established with the continuous method and with the discontinuous method with the quinhydrone electrode was pointed out. The late acid effects following injection of cyanide, as indicated by the quinhydrone electrode, were at times considerably greater than those recorded with the manganese dioxide electrode. It was suggested that the relatively alkaline values yielded by the manganese dioxide electrode following massive doses of cyanide were due to the formation of metabolites which on reaching the electrode exerted a specific effect. Therefore, "Precaution against the effects of these reducing substances was stressed" (Gesell and Hertzman, 1926). It was pointed out that "Unfortunately, the quinhydrone electrode is also an oxidation-reduction electrode." "That it will be a sound precaution to use the hydrogen electrode as the final check in experimental work in which reducing substances may vary as a result of disturbed metabolism. We hope to avoid misinterpretation of extraneous potential changes by observing this precaution and perhaps evaluate the significance of oxidation-reduction phenomena in respiratory control."

In these experiments we have used the precaution of checking the manganese dioxide, the quinhydrone and the hydrogen electrode against one another. This was done in eight experiments, of which figures 1A, B and C, 3A, B and D, and 4A and B are examples. In each of these the manganese dioxide potential changes are continuously recorded and the quinhydrone and hydrogen electrode acidity curves are established by selected blood samples which are plotted on the original records. The blood was drawn into heparinized saline solution and immediately centrifuged. The pH determinations were done on the plasma, first with the hydrogen electrode and then with the quinhydrone electrode. In the three experiments represented in figures 1A, B and C the blood samples were sufficiently large (50 cc.) to permit three to four determinations with the hydrogen electrode and four to five were done with the quinhydrone electrode. As a double check, one of us (A. B. H.) made the hydrogen electrode determinations and the other (R. G.), the quinhydrone electrode determinations.

The impairment of tissue metabolism was severe particularly in figures 1B and C. In 1B the mean blood pressure fell to nearly zero and in 1C the circulation failed entirely and a sluggish flow of blood was maintained by massage for thirty-five minutes. In figure 1C the degree of impaired oxidations indicated in the record of artificial ventilation would seem to offer ample opportunity for the accumulation of reducing metabolites. It will be seen that the hydrogen and quinhydrone electrodes agreed with each other within the limits of error and that both disagreed with the manganese dioxide electrode. The sharp "alkaline" bend of the manganese dioxide curve occurring during an acid change, as indicated by

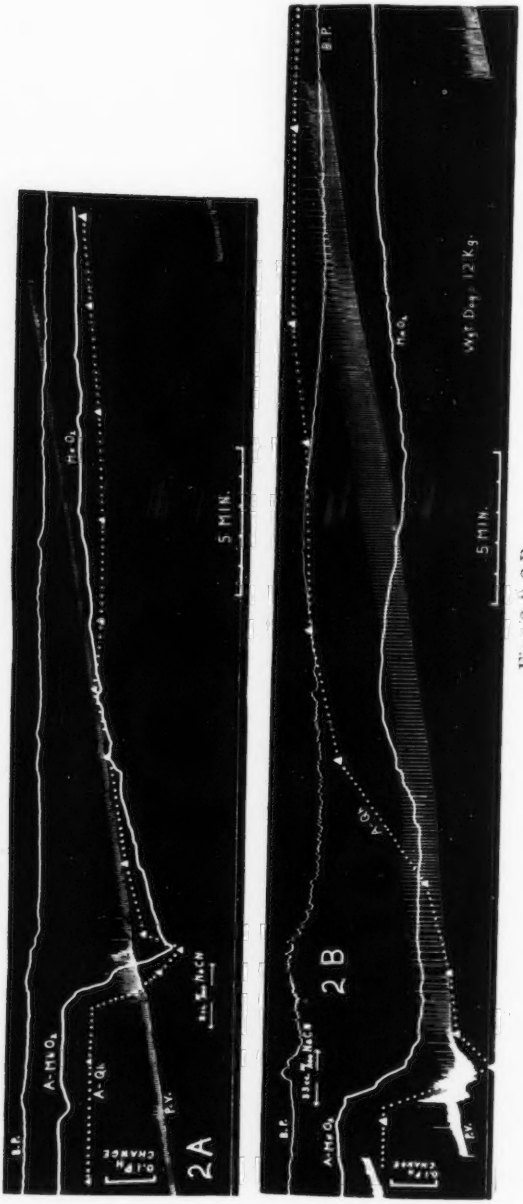
1A
A-M₂O₂
A-Q₂
A-H₂
Wgt. Dog = 11 Kg
5 MIN.
0.1 FM CHANGE
1B
A-H₂
A-Q₂
Wgt. Dog: 13.5 Kg
A-M₂O₂
5 MIN.
0.1 FM CHANGE
1C
A-H₂
A-Q₂
A-M₂O₂
Wgt. Dog = 12.7 Kg
5 MIN.
M₂O₂
Q₂
0.1 FM CHANGE

Fig. 1

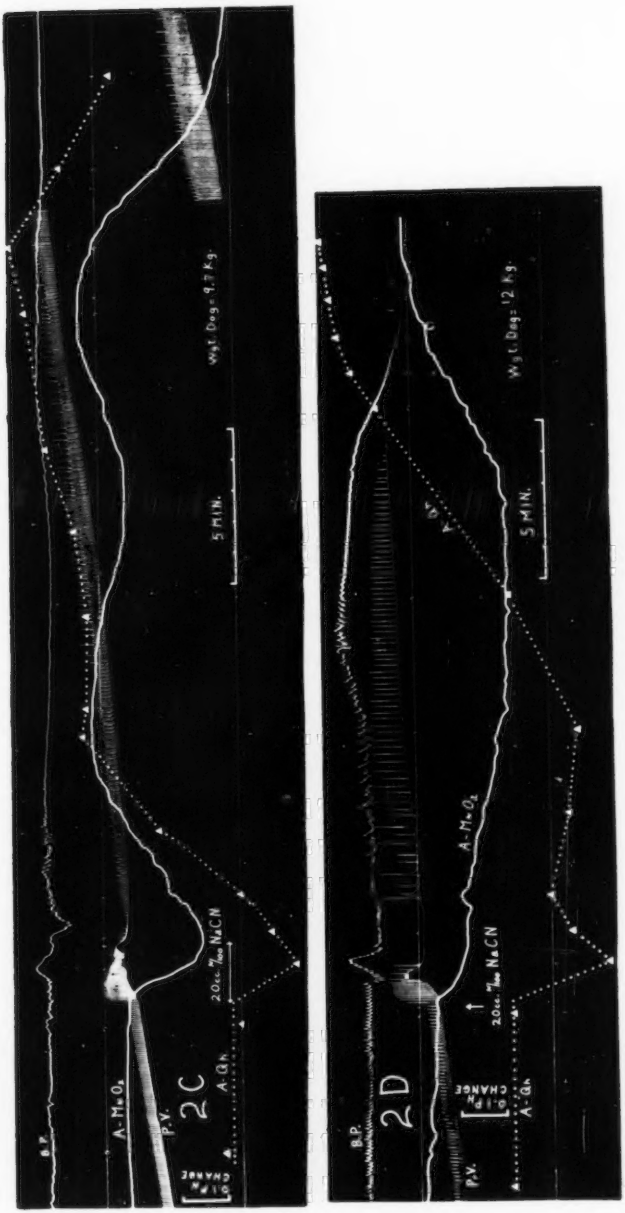
the hydrogen and quinhydrone electrode, suggests the appearance of reducing metabolites in the circulating blood and emphasizes the precaution that we pointed out.

But these experiments had a second purpose: that of indicating the reliability of the quinhydrone electrode for the determination of pH changes during severe metabolic disturbances. The rapidity of the method and the small amount of blood required for several determinations highly recommend the substitution for our type of problem of this electrode for the hydrogen electrode. It is, of course, conceivable that any electrode might be influenced by the chemical changes in the blood resulting from changes in metabolism. But assuming that the hydrogen electrode gives reasonably reliable information on the pH changes under the conditions supplied, the validity of the quinhydrone electrode under like conditions is indicated. Further data on this point are found in figures 3A, B and D and figures 4A and B. These experiments were planned to provide not only data on the comparative behavior of the quinhydrone electrode but to provide at the same time information on the effects of sodium cyanide on changes in blood acidity. It was, therefore, necessary to avoid the effects of hemorrhage. The blood samples were consequently reduced to the minimum amount required for one pH determination with the hydrogen electrode. The results, in general, indicate that if the quinhydrone electrode is affected by the presence of reducing substances in the plasma, the effect is small or similar in degree to modified potential changes occurring at the hydrogen electrode.

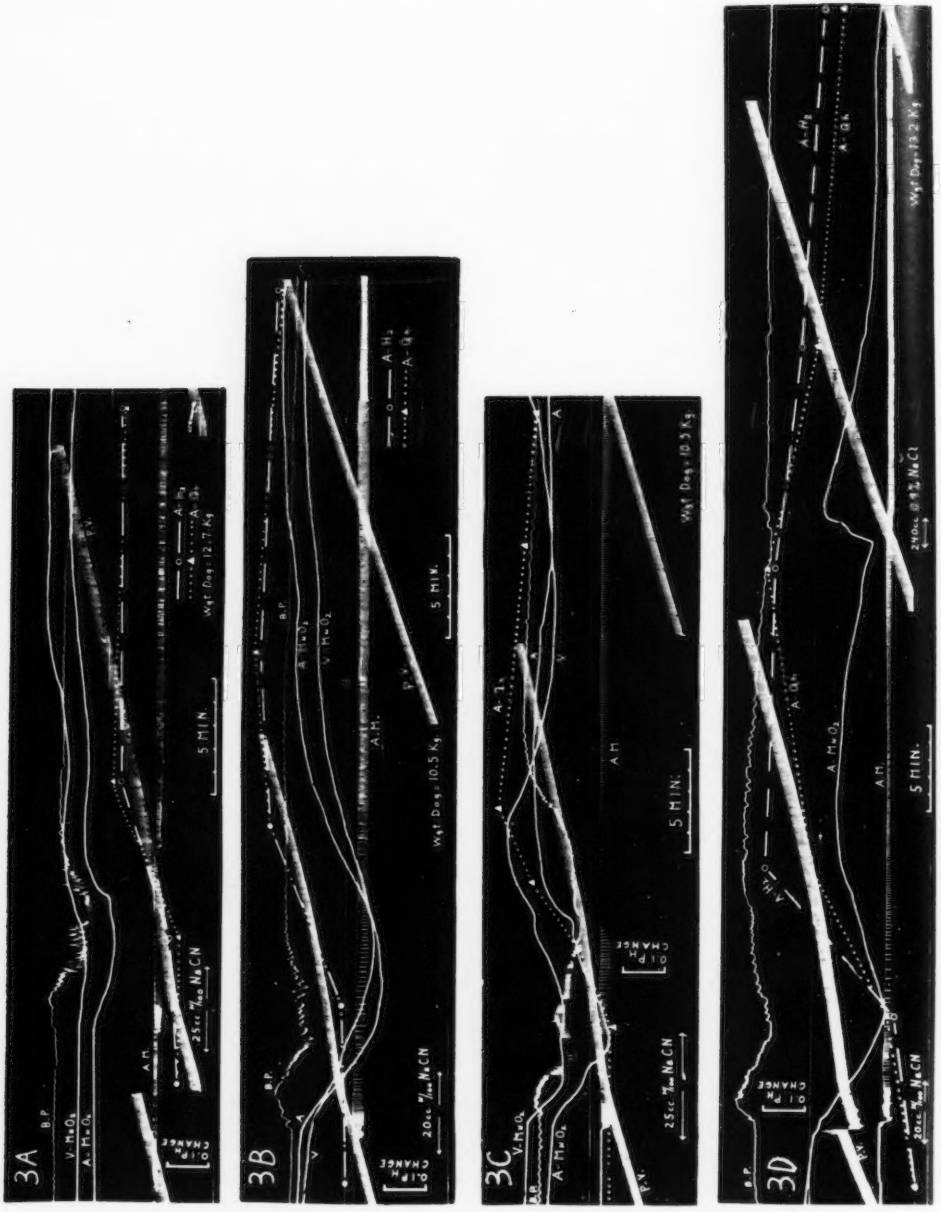
Figures 2A, B, C and D show effects of short and rapid administrations of N/100 sodium cyanide during normal ventilation on the manganese dioxide electrode in the circulating arterial blood and on changes in acidity in discontinuous samples, as indicated by the quinhydrone electrode. Where slight differences in acidity are obtained on the two pre-injection blood samples which fall within the limit of error of the method it is assumed that no significant change in acidity has occurred, and the curve is projected horizontally between these initial points. Otherwise the discontinuous curves are straight line curves drawn point to point. These four records in figure 2 are taken from four different animals. Significant variations in response attributable to dosage and characteristics of the animal appear. In all of the records there is an initial increased alkalinity which is definite and pronounced. The later acid change is more variable. In figure 2A with a small injection of only 8 cc. sodium cyanide an extensive initial alkaline swing is followed by a very slow and progressive acid change, which at the end of approximately twenty-eight minutes reaches an acid level only 0.05 pH above the initial pre-injection level. Apparently the proportion of anaerobic to aerobic metabolism was not markedly changed. Compare these results with those of figure 2B in which 33 cc.


B.P.
A-Mu Qs
A-Qk
P.V.
0.1 P.M. CHANGE
2A
M-Qs
5 MIN.
B.P.
A-Mu Qs
2B
A-Qk
P.V.
0.1 P.M. CHANGE
M-Qs
Wgt Dep. 1.2 Kg.
5 MIN.

Figs. 2 A, 2 B


B.P.
A-M.O2
P.V.
2C
A-Qh
0.1 Pa CHANGE
20cc. 1/100 NaCN
Wgt. Dog: 9.7 Kg.
5 MIN.
B.P.
2D
A-M.O2
P.V.
0.1 Pa CHANGE
A-Qh
20cc. 1/100 NaCN
Wgt. Dog: 12 Kg.
5 MIN.

Figs. 2C, 2D


3A
B.P.
V. H_2O_2
A- H_2O_2
A.M.
P.I.
O — A- H_2
▲ ····· A- O_2
Wgt Dog - 12.7 Kg
5 MIN.
25cc 7‰ NaCN
DIP IN CHANGE
3B
B.P.
A- H_2O_2
V. H_2O_2
A.M.
P.I.
O — A- H_2
▲ ····· A- O_2
Wgt Dog - 10.5 Kg
5 MIN.
20cc 7‰ NaCN
DIP IN CHANGE
3C
V. H_2O_2
B.P.
A- H_2O_2
P.I.
A- H_2
A.M.
Wgt Dog - 10.3 Kg
5 MIN.
25cc 7‰ NaCN
DIP IN CHANGE
3D
B.P.
P.I.
A- H_2
A- O_2
A- H_2O_2
A.M.
Wgt Dog - 13.2 Kg
5 MIN.
240cc @ 1% NaCl
20cc 7‰ NaCN
DIP IN CHANGE

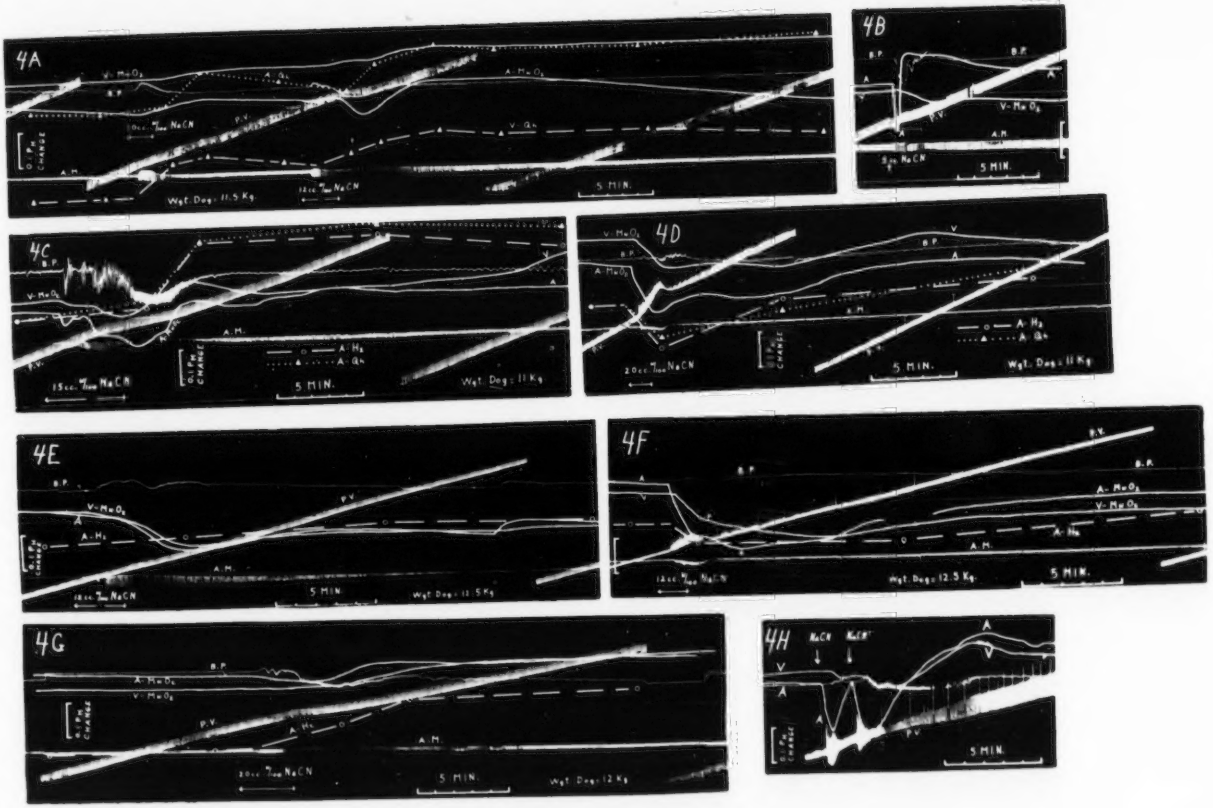


Fig. 4

of sodium cyanide were injected and in which oxidations (as indicated by the respiratory record, *P. V.*) are markedly reduced. The acid swing is larger and decidedly more abrupt despite the fact that pulmonary ventilation was enormously increased. A disproportionate anaerobic acid metabolism with diffusion of fixed acid into the circulating blood increasing the acid effects of free carbonic acid fits the picture of acid changes occurring in the blood. Comparable results were obtained in figure 2C where hyperacidity again developed in the presence of increased pulmonary ventilation. Of interest is the secondary period of increased pulmonary ventilation associated with marked increased alkalinity of the blood of about 0.3 pH. Contrast these final changes in acidity with those of figure 2D, where a progressive increasing acidity prevails as respiratory failure develops.

In figures 3A, B, C and D and 4A, C, E and G effects of sodium cyanide on the potential of the manganese dioxide electrode in the circulating arterial and venous blood are shown during the administration of constant artificial ventilation during pneumothorax. Arterial acidity curves established with the hydrogen and quinhydrone electrodes are plotted on the records. Comparison of the manganese dioxide curves in figures 3 and 2 shows considerable resemblance—an initial alkaline swing followed by increasing acidity. But comparing the curves established by the hydrogen and quinhydrone electrodes the initial alkaline swing associated with increased respiratory movements with the chest intact is missing in the experiments with constant artificial ventilation. The later increased acidity, however, is retained. This constitutes a constant difference between the effects of cyanide during normal and constant artificial ventilation, as more satisfactorily illustrated in figures 4C and D and 4E and F. Here sodium cyanide is administered under both conditions in each of two dogs—first during pneumo-thorax with constant ventilation and second after closure of the thorax and with normally controlled ventilation.

Obviously the initial increased alkalinity of the arterial blood during normal ventilation is a result of increased pulmonary ventilation for that is missing when ventilation does not change. And since the later increased acidity appears during constant artificial ventilation or increasing normal ventilation it is presumably of metabolic origin. To be sure the final alkaline recovery with the chest intact is most naturally attributed to the augmented ventilation, yet comparable alkaline recovery obtaining in figures 3A, B, C and D, though ventilation remains constant, is suggestive of the influence of metabolic and excretory factors such as the oxidation and excretion of fixed acids.

In figures 5A, B and C the effects of sodium cyanide during normal ventilation on changes in acidity of the arterial and venous blood, as estabblished with the quinhydrone electrode, are shown. Arterial and venous

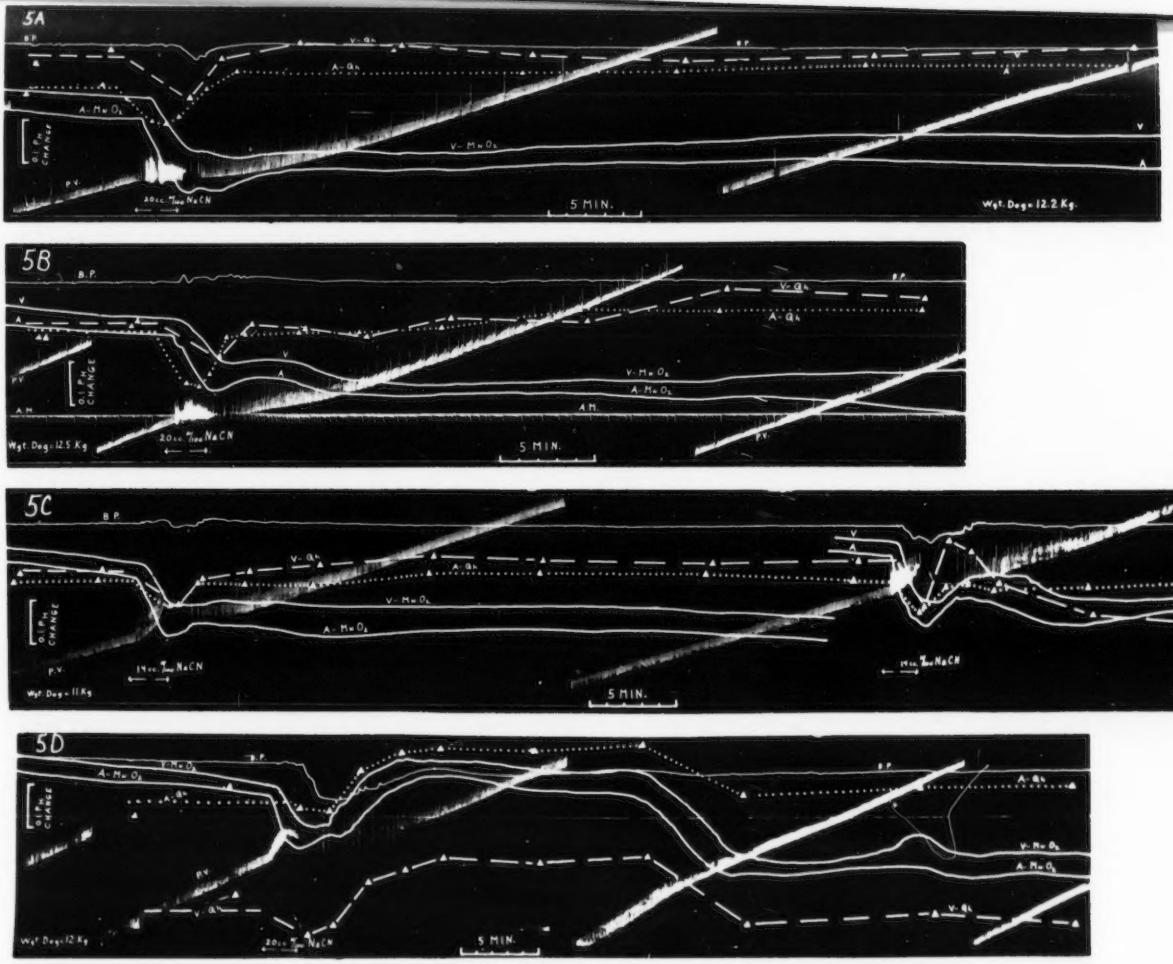

5A
B.P.
V-Qk
A-Qk
A-Mw O2
V-Mw O2
O.I.P.M. CHANGE
P.V.
20cc 1/100 NaCN
5 MIN.
Wgt. Dog = 12.2 Kg.
5B
B.P.
V
A
V-Qk
A-Qk
V-Mw O2
A-Mw O2
A.M.
O.I.P.M. CHANGE
P.V.
Wgt. Dog: 12.5 Kg
20cc 1/100 NaCN
5 MIN.
5C
B.P.
V-Qk
A-Qk
V-Mw O2
A-Mw O2
O.I.P.M. CHANGE
P.V.
Wgt. Dog = 11 Kg
14cc 1/100 NaCN
5 MIN.
5D
B.P.
A-Mw O2
A-Qk
V-Mw O2
A-Mw O2
O.I.P.M. CHANGE
P.V.
V-Qk
Wgt. Dog: 12 Kg
20cc 1/100 NaCN
5 MIN.

Fig. 5

manganese dioxide curves are registered as well. The usual changes in acidity of the arterial blood illustrated in figures 2A, B, C and D, and 4D and F occur. The new and striking point of these records is the similarity of the acid changes in the arterial and venous blood. This seems to hold in general. An exception, however, is noted in figure 4B, in which acid changes are recorded during constant artificial ventilation. The injection is rapid and the effects are abrupt. The arterial blood swings sharply alkaline and the venous blood temporarily but barely noticeably more acid. The arterial blood next turns hyperacid and the venous blood slightly more alkaline. These effects are believed to be due to sudden changes in volume-flow of blood, for it will be noted that they are not unlike those produced by hemorrhage and reinjection. The sharp fall in blood pressure is undoubtedly associated with a decreased volume-flow and the subsequent return of blood pressure is probably accompanied by an increased flow of blood, for it is known that cyanide produces an increased volume-flow (Bernthal, Bronk, Cordero and Gesell, 1928.) It is, therefore, suggested that altered flow of blood is a contributing factor to changes in blood acidity produced by sodium cyanide. This phase of the subject, however, is reserved for future experiments in which changes in volume-flow of blood will be recorded along with changes in blood acidity.

Though with normal ventilation the manganese dioxide electrode and the hydrogen and quinhydrone electrodes show directional agreement and in some experiments fairly close quantitative agreement, a distinct and invariable discrepancy developed on the administration of cyanide during constant artificial ventilation. Whereas the acidity curves established by the hydrogen and quinhydrone electrodes regularly deflect in the acid direction, the manganese dioxide curve deflects in the alkaline direction. This we have taken (Gesell and Hertzman, 1926) and still consider as an index to an increased liberation of reducing metabolites. Figures 3B, C and D are most satisfactory in this respect for they show large changes in potential—0.2–0.3 volt with virtually the same pH. Bishop and West (1926) have demonstrated under these conditions of constant pH that the lead peroxide electrode is satisfactory for determining oxidation-reduction potentials.

We are not prepared to suggest what substances may be producing this reducing effect upon the electrode. Neither do we care for the present to consider the significance of the appearance of these substances in the blood with respect to respiratory control. But it is of interest that a change in potential at the manganese electrode occurs so promptly after the administration of cyanide independent of acid change. We are reminded of the views of Pflüger on the rôle of reducing substances in the circulating blood on respiratory control.

In passing it might not be amiss to record an impression which we as yet have not had time to verify; namely, that metabolic disturbances produced by cyanide seem to elicit discrepancies between the manganese dioxide potential changes and those occurring at the hydrogen and quinhydrone electrodes more readily than other metabolic disturbances, such produced by the administration of low oxygen. This suggests that the action of cyanide is more inclusive than the simple impairment of the oxidative mechanism of the cell.

Discussion. The mechanism producing the changes in pulmonary ventilation elicited by cyanide may now be considered in relation to changes in oxidation and changes in acid relations at the respiratory center. If a diminution in rate of oxidation exerts a stimulating effect on pulmonary ventilation the initial increased ventilation is most obviously accounted for. (The respiratory record indicates an increased oxygen consumption which is undoubtedly explained by an increased inspiratory position of the chest.) But for a short period following this augmented pulmonary ventilation respiration is depressed though the rate of oxidation is still considerably below normal. This we believe is a result of rapid administration of sodium cyanide and is due to exceedingly pronounced depression of oxidations. It is conceivable that temporarily the energy necessary for rhythmic discharge may not be available. For, if, as we have more recently found, a more dilute solution is injected at a slower rate pulmonary ventilation may be progressively increased. Probably under such conditions no period of acute depression of oxidation is reached. The need of oxidation for respiratory discharge is further suggested in our present experiments by the fact that respiratory movements return with the return of oxidations. Not only do they return but they continue to augment as oxidations increase. This behavior is in apparent contradiction to the inverse relation between pulmonary ventilation and rate of oxidations.

Similar difficulty is encountered in aligning the changes in pulmonary ventilation with changes in acidity of the blood. Take, as the clearest example, figure 2B. Pulmonary ventilation is augmented during a rapid increase in alkalinity of the blood immediately following injection of cyanide but later it is decidedly above normal though the blood acidity is definitely greater than normal. The common association of increasing alkalinity of the arterial blood and increasing pulmonary ventilation under a variety of conditions led us in a preceding paper (1927) to analyze the causal relation between pulmonary ventilation and acidity of the blood with changing alveolar oxygen pressure. In that paper it was pointed out that the acidity of the immediate external environment of the respiratory neurones might follow the changes in acidity of the circulating blood more closely than of the tissues. Increased pulmonary ventilation with the administration of low oxygen might, therefore, conceivably be due to

an increased alkalinity of the immediate cell environment. The increased pulmonary ventilation noted in figure 2 obviously could be accounted for in the same way. In that connection, however, the experiments in figure 3 are of special interest for here sodium cyanide elicits increased pulmonary ventilation though now the blood initially turns acid. The experiment demonstrates at least that if the external environment varies in acidity with the blood that the acidity of the environment is not the predominant controlling factor.

Whether or not the changes in acidity inside the cell are significant hinges on the evidence for or against increasing acidity of the tissues resulting from cyanide administration. The fact that the blood invariably turns more acid with constant ventilation indicates altered acid metabolism leading to increasing acidity of the cells. But the fact that the blood may turn more alkaline with administration during normal ventilation suggests the possibility that the tissues themselves may turn more alkaline under these conditions. The same reasoning was used to test the acid mechanism of control of ventilation with the administration of low oxygen, but it was concluded on analysis of the data that low alveolar oxygen probably led to increased acidity of the tissues though the blood turned more alkaline.

It might be assumed, for example, that with the sudden cessation of ventilation following the initial excitatory stage in figures 2 and 5, the tissues had become more alkaline as a result of increased washing out of carbon dioxide, but the same depression occurs in the experiments with constant artificial ventilation. The depression, then, seems best explained, as pointed out before, by excessive reduction in oxidations. To be sure, some of the records with normal ventilation taken by themselves give undecisive evidence for increased tissue acidity for the post-administration acidity of the blood is not very much greater than the pre-administration acidity. See, for example, figures 3A, B and C and 4D and F. Yet all show a final greater acidity. This, supported by the more striking examples of pronounced increasing acidity of the blood in the presence of equally pronounced increasing pulmonary ventilation, speaks in favor of increased tissue acidity. Why one animal should develop greater acidity than another despite the effect of enormously increasing ventilation (see fig. 4H) we cannot say for lack of definite information. Figure 4H suggests a rapid outpouring of fixed acid, the effects of which are not compensated by hyperventilation.

SUMMARY AND CONCLUSIONS

The effects of rapid intravenous injection of N/100 sodium cyanide on the acidity of the circulating arterial and venous blood of the dog was studied with the manganese dioxide, hydrogen and quinhydrone electrodes.

A comparison of the behavior of these electrodes during severe metabolic disturbances produced by impaired circulation and by cyanide administration showed good agreement between the hydrogen electrode and the quinhydrone electrode. The manganese dioxide electrode registered progressively increasing alkaline values during impaired metabolism.

On the assumption that the hydrogen electrode was not affected by variations in metabolites in the blood plasma obtaining in these experiments, it was further assumed that the quinhydrone electrode gave relatively satisfactory results under the conditions obtaining in our experiments. The discrepancy on the part of the manganese dioxide electrode was attributed to the chemical effects of reducing metabolites liberated by the tissues during impaired metabolism.

During normally controlled ventilation with the chest intact, initial increasing alkalinity of the arterial and venous blood followed by progressively increasing acidity above the preinjection level were indicated by both the hydrogen and quinhydrone electrodes. The final increased acidity varied from approximately 0.04 pH to 0.4 pH.

With constant artificial ventilation during pneumo-thorax the initial increased alkalinity was missing and was replaced by increased acidity of the blood.

The initial increased alkalinity of the blood during normal ventilation with the chest intact was attributed to excessive ventilation of the lungs. This conclusion was supported by the absence of the alkaline effect with constant artificial ventilation.

The secondary increased acidity of the blood with both types of ventilation was explained by a disproportionate increase in anaerobic acid metabolism over aerobic acid metabolism. Fixed acids leaving the tissues in greater abundance reduce the buffer base of the blood and increase the acid effects of carbonic acid. This coupled with the acid effect of a more highly oxidized hemoglobin and with a broken coördination of the dual function of hemoglobin from a more or less permanently oxidized condition of the hemoglobin leads to increasing blood acidity.

The primary effect of rapid administration of cyanide was stimulation, and then depression of respiratory movements during acute impairment of oxidation, followed by progressively augmented movements ultimately giving way to recovery during the period of improved oxidations. The ventilatory response as indicated by abdominal movements was approximately the same during constant artificial ventilation and during normal ventilation with the chest intact.

An attempt to align changes in pulmonary ventilation with associated phenomena indicate better agreement with changing acid relations than with changes in oxidative rate.

The fact that pulmonary ventilation with the chest intact increased

during the initial stage of increasing alkalinity of the blood and later during increasing acidity of the blood suggests that neither the acidity of the blood nor the immediate cell invironment is the predominating factor controlling ventilation. This is further supported by the fact that pulmonary ventilation is increased by cyanide during constant ventilation though the acidity of the blood may temporarily show no increase or only a small increase in acidity.

The probability of increased cellular acidity resulting from disturbed oxidations and impaired acid transport supports the view of the significance of intracellular acidity as a factor in control of ventilation.

BIBLIOGRAPHY

BISHOP, G. H. AND A. WILLIAMS. 1926. Proc. Soc. Exper. Biol. and Med., xxiv.
GESELL, R. 1925. Physiol. Reviews, v, 551.
GESELL, R. and A. B. HERTZMAN. 1926. This Journal, lxxviii, 206.
1927. This Journal, lxxxii, 591.
BERNTHAL, BRONK, CODERA AND GESELL. 1928. This Journal, lxxxiii, 435.

THE REGULATION OF RESPIRATION

XVIII. The Effects of Low and High Alveolar Oxygen Pressure and of Sodium Cyanide on the Carotid and Femoral Flow of Blood as Studied with the Continuous Electrometric Method

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Received for publication October 3, 1927

In a preceding paper (Bronk and Gesell, 1927), effects of administration of carbon dioxide on the carotid and femoral flow of blood were described. In the present paper we discuss the effects of changing alveolar oxygen pressure and of the administration of sodium cyanide. The purpose of these studies is an attempt at a closer correlation of and determination of the mechanism of circulatory and respiratory control. The need of an adequate volume-flow of blood for normal gaseous metabolism hardly calls for more comment than the need of adequate pulmonary ventilation, yet the mechanism by which the flow of blood is controlled and adjusted to varying conditions at the lungs and in the tissues warrants further study. As a single example of the close interdependence of circulation and ventilation, attention is momentarily called to the rhythmic changes in mean blood pressure, carotid flow of blood and pulmonary ventilation—all elicited by administration of a 7 per cent gaseous mixture of oxygen in figure 1A.

In our first experiments on circulatory control (Bronk and Gesell, 1927), it was found that carbon dioxide increased the carotid flow and decreased the femoral flow of blood. Since these changes occurred whether mean blood pressure was maintained at a constant level or allowed to fluctuate, the changes in flow were attributed to independent vasomotor effects occurring in the head and leg regions. To what extent these effects are of central or peripheral origin was not determined but the possibility of

[1] Reported in the Proc. Soc. Exper. Biol. Med., 1926, xxiv, 257.

This investigation has been made with the assistance of a grant from the committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

These experiments were begun by Detlev W. Bronk and Robert Gesell and completed by Theodore G. Bernthal, who did most of the experiments. Narciso Cordero, National Research Fellow, participated in some of the later experiments.

associated central chemical control of circulatory and ventilatory activity was postulated. It was concluded that "The reciprocal relation between carotid and femoral flow suggests that circulatory adjustments following variations in carbon dioxide content of the body occur in favor of the brain." These results and conclusions may have a bearing on the results of varying alveolar oxygen pressure and of administering sodium cyanide, for low oxygen and cyanide not only affect the rate and state of oxidation but alter acid relations as well.

METHOD. The methods were similar to those employed in the carbon dioxide experiments. Thermopile vessels were inserted in the carotid and femoral arteries of heparinised dogs and the flow mechanically recorded with Leeds and Northrup type K potentiometers. Gaseous mixtures were administered with rebreathing tanks by normal ventilation with the chest intact and by constant artificial ventilation during pneumo-thorax. Time was recorded in seconds and five-second intervals.

RESULTS. Typical effects of administration of gaseous mixtures low in oxygen are seen in figure 1C in which the administration of room air is temporarily interrupted by 6, 12 and 8 per cent mixtures of oxygen. The carotid flow increases and on readministration of room air diminishes. Opposite effects occur in the femoral flow, an initial decreased flow gives way to increased flow. (The large augmented flow following late on the readministration of room air after 12 per cent oxygen is the result of intravenous injection of glucose solution.) The general downward drift common to figure 1C and others is an index to progressive deterioration of circulation common to our experimental condition. A more accurate interpretation of results is, therefore, obtained by visualizing the basal flow drift.

As was the case in the experiments (Bronk and Gesell, 1927) on the effects of carbon dioxide on volume flow of blood, the changes in flow though influenced by blood pressure may occur independent of blood pressure change (see figure 2E). The carotid flow increases with low oxygen though the mean blood pressure was maintained at a constant level.

In figure 1F effects of intravenous injection of sodium cyanide are shown. There is a marked increased carotid flow giving way during recovery to a gradual return and the opposite changes occur in the femoral artery: an initial decreased flow giving way to an increased flow during recovery.

In the carbon dioxide experiments it is safe to assume that on the administration of carbon dioxide the tissues act as buffers to the blood by virtue of the passage of carbon dioxide from the blood to the tissues. The direct experiments of Gesell and Hertzman (1926) demonstrating the alkaline change occurring in the carbonated blood as it passes through the tissues is indicative of a reciprocal acid change occurring in the tissues. Assuming a similar acid change within the tissues occurring as a result of

lowered alveolar oxygen and injection of sodium cyanide, the directional changes in carotid and femoral flow of blood might likewise be interpreted on an acid basis. A careful analysis of the changes in the femoral flow, however, suggests that this explanation may need further analysis. For it will be noted that although all of the femoral records show an initial decrease in volume-flow of blood on the administration of low oxygen, if the administration is prolonged enough decreased flow eventually gives way to increased flow. This is well illustrated in figures 1C, D, G and H and 2B, D, F and G. Not only may the flow increase but it may reach a level considerably above the pre-administration flow. For example, see figures 2B and 2D. And added to this increase there is a common and sharp post-administration increase following immediately on breathing of room air. This final increase, then, gives way to recovery.

A glance through the records will show a great variability in the femoral response to low oxygen. The initial decrease in flow may be large and prolonged, as in figure 3D, or small and of short duration, as in figure 1G. The increase in flow during administration may be large or small (figs. 2D and 1G) and similarly the post-administration increase may show big differences as in figures 3G and 3E. These same variations in response may be noted on the administration of sodium cyanide (see figs. 3A, 1B, and 1F). Here they appear in the carotid flow as well as in the femoral flow.

In figures 2 and 3 effects of 50 and 100 per cent oxygen mixtures on carotid and femoral flow are shown. Figures 2A and B are taken from the same animal. In figure 2A high oxygen is seen to reduce both the carotid and femoral flows. On re-administration of room air the volume-flow of blood recovers. In figure 2B high oxygen produced the same effect though volume-flow had just been augmented by intravenous injection of dextrose solution. With re-administration of room air there is a tendency to recover. Full recovery is undoubtedly prevented by the rapidly decreasing flow of blood which always follows augmented flow from increased blood volume. The final administration of a 10 per cent oxygen mixture brings out the opposite effects of decreasing the alveolar oxygen pressure. Figures 2C and 2D are taken from another animal. A 100 per cent oxygen mixture leads to decreased carotid and femoral flow with recovery on readministration of room air; and the 7 per cent oxygen mixture produces a general increase in carotid and femoral flow of blood with recovery on readministration of room air. The diametrically opposite results of high and low oxygen are shown again in figure 2F from a third dog. In figure 3A the effects of high oxygen and sodium cyanide are compared and found to be opposite. Our general results on the effects of alveolar oxygen pressure have recently been confirmed by Tivel (1927), who found by direct inspection of the superficial vessels of the brain that administration of oxygen reduces the calibre of these vessels.

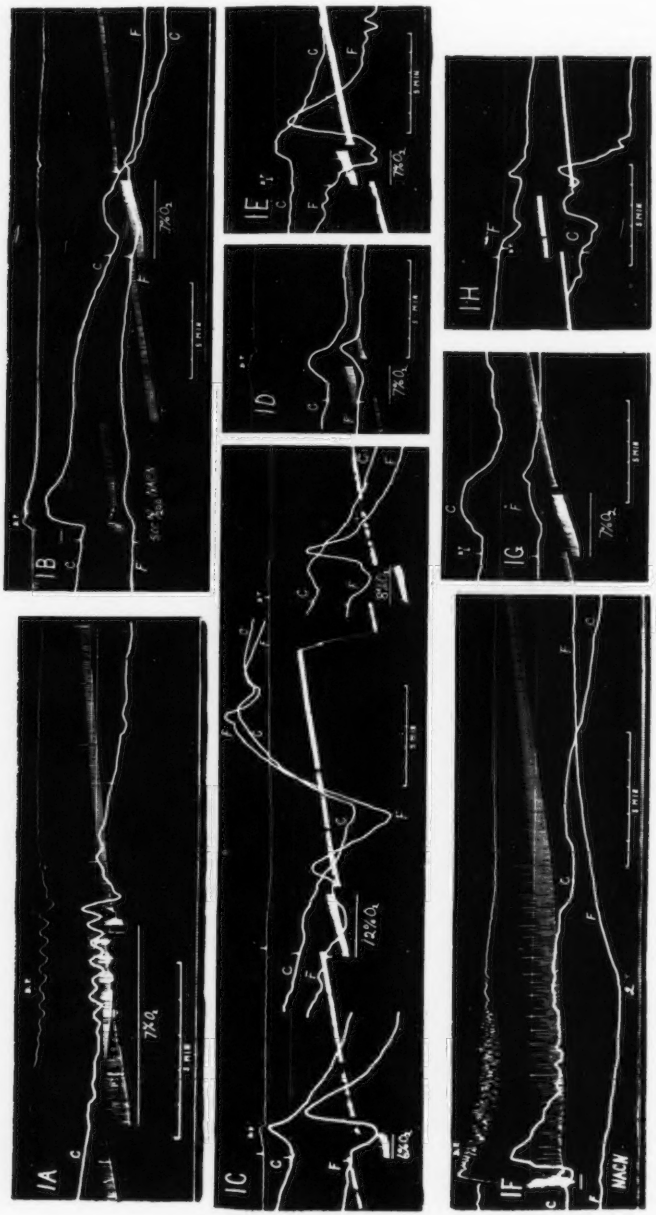

Fig. 1

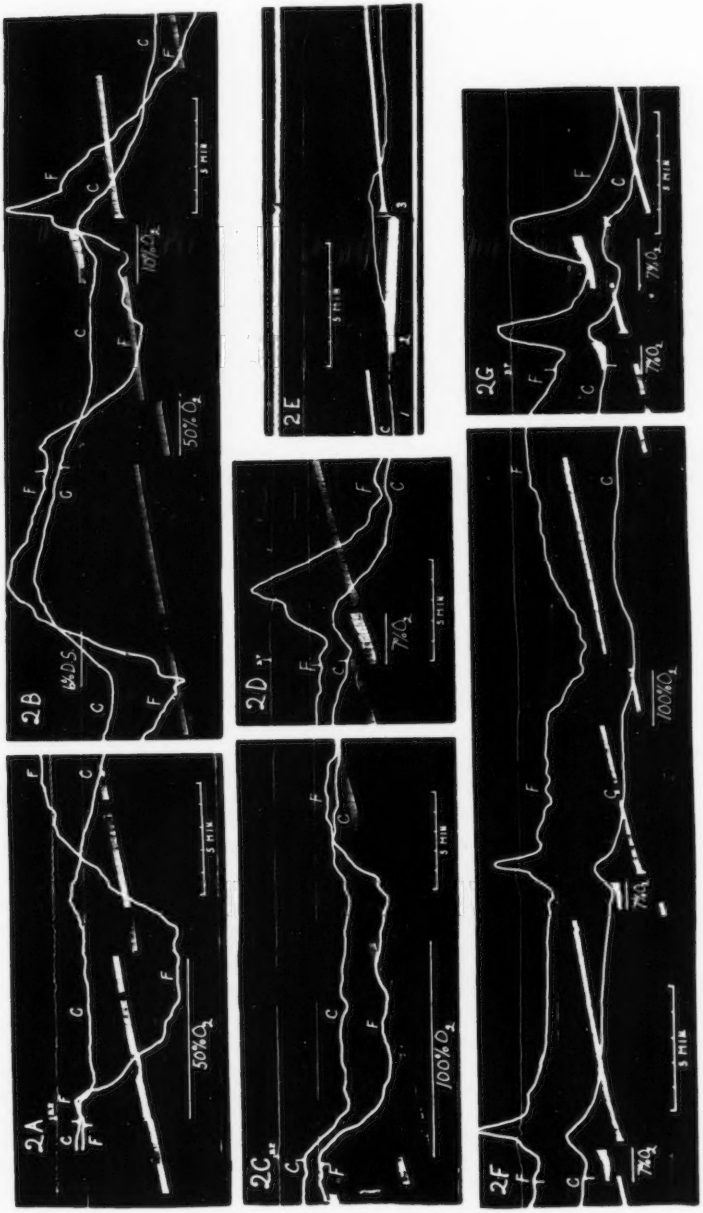
2A
50% O2
5 MIN
2B
6% DS
100% O2
50% O2
5 MIN
2C
100% O2
5 MIN
2D
7% O2
5 MIN
2E
5 MIN
2F
7% O2
100% O2
5 MIN
2G
7% O2
7% O2
5 MIN

Fig. 2

As was pointed out before, the carotid curve of flow on administration of low oxygen is usually a simple curve. It, therefore, is not surprising to find a simple but inverse curve on administration of high oxygen. The femoral curve of flow elicited by low oxygen, however, is compound. An inspection of the high oxygen curves suggests that the initial decreased and increased flow of blood produced by low oxygen have their counterpart in the increased and decreased flow respectively in the administration of high oxygen. For in figures 2A and 2B and 3A the decreased flow is preceded by a transitory increased flow.

Without specially devised experiments it is hazardous to venture an explanation of the difference in behavior of the carotid and femoral flow of blood to the administration of carbon dioxide, low oxygen and cyanide. A dual peripheral and central acid mechanism of vasomotor control, however, is recalled (Gesell, 1923). In experiments on the volume-flow of blood through the left and right submaxillary gland, one with the innervation intact and the other with the innervation severed, it was found that the final effects of intravenous injection of acid differed in the two glands. On the denervated side volume-flow was augmented, on the intact side it was reduced. Whether relatively comparable conditions obtain in the head and leg regions in our present experiments is purely a matter of speculation. Wiggers has demonstrated the presence of vasomotor nerves in the brain of the dog. Nevertheless it is within the range of possibility that in the carotid flow we are dealing primarily with the peripheral dilating effects of acid and in the femoral flow with a resultant of a more active central constricting effect and of peripheral dilating effects. Thus the central constricting effect coming first into evidence could give way to a peripheral dilating effect. The post-administrational increase in femoral flow could be accounted for by the same reasoning—a dilating effect of a suddenly increased alkalinity of the center and the subsequent decreased flow to increasing alkalinity at the peripheral mechanism. It is interesting to recall in this connection that although carbon dioxide produces a long maintained decrease in the femoral flow, readministration of room air leads first to a very rapid increase in volume-flow of blood considerably above the pre-administration flow which then returns to normal.

The explanation of the volume-flow changes should not be pressed too much at this point, yet the value of our suggestion is related to a final explanation of the difference in flow in the carotid and femoral arteries produced by the administration of carbon dioxide and low oxygen. In the carbon dioxide experiments the results were tentatively interpreted on the basis of the assumption that the carotid flow indicated the brain flow. It was stated that "The difficulty of isolating the cerebral flow has been a hindrance to many problems. The continuous method, however, seemed to offer a crude approach for following changes in cerebral

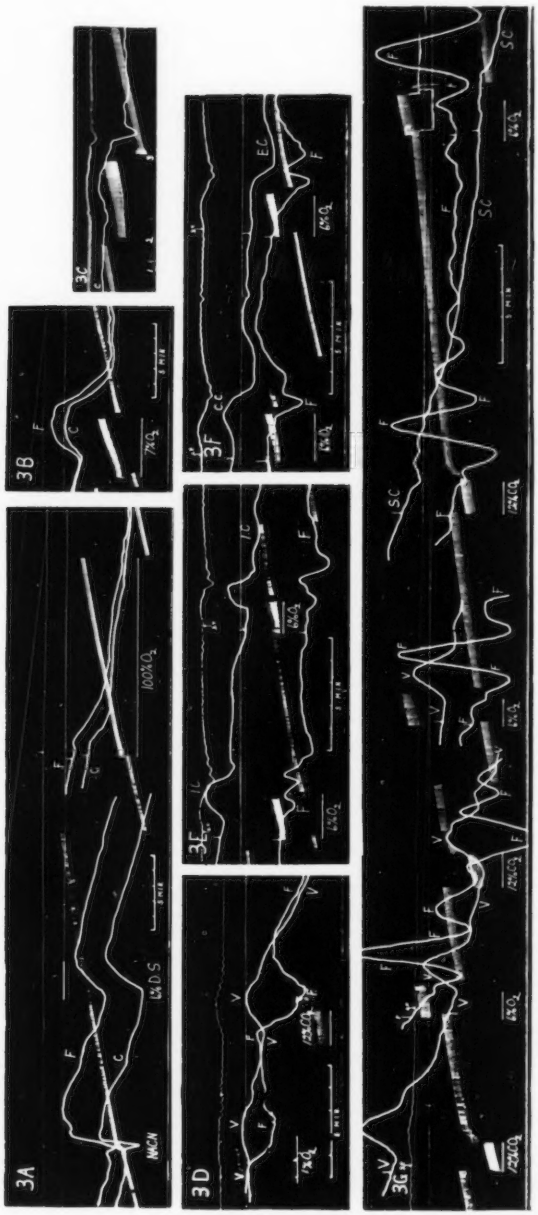

Fig. 3

flow by recording the carotid flow. Though the carotid artery carries blood to skin, muscle, gland and bone the cerebral flow may predominate. If, for example, a constant difference is noted in the carotid and femoral flows on the administration of carbon dioxide, it might not be unreasonable to attribute this difference to the effects of cerebral flow. On this assumption the carotid and femoral flows were recorded to follow changes in blood-flow distribution under a variety of conditions."

In these experiments this assumption was put to the test by isolating and recording the vertebral flow of blood. In figure 3D the effects of administration of a 6 per cent oxygen mixture and a 12 per cent carbon dioxide mixture by constant artificial ventilation are shown. The results are clean cut and striking. The femoral flow shows prolonged reduction both with low oxygen and with carbon dioxide and the vertebral flow shows a decided increase with both administrations. In figure 3G the experiment is repeated on another animal and compared with results on the subclavian flow. First the effects of 12 per cent carbon dioxide on vertebral flow are shown. The changes in flow are exceedingly abrupt. With the next administration, 6 per cent oxygen, the changes in femoral flow are also recorded. The changes in the vertebral flow are as abrupt as with the administration of carbon dioxide. The usual decrease in femoral flow is followed by an exceptionally large increase in flow. (The record is complicated by rhythmic oscillations in flow. On the whole these are more frequent in the femoral than in the carotid artery.) The effects of carbon dioxide and low oxygen are next repeated with the same results. The final two observations show the flow in the subclavian artery with the vertebral arteries occluded first in the administration of carbon dioxide and next in the administration of a 6 per cent oxygen mixture. The relatively small increases in subclavian flow certainly stand in marked contrast to the changes in flow recorded in the vertebral artery. Why the subclavian flow differs from that of the femoral we cannot definitely say. It may be related to the fact that the artery was temporarily occluded before the effects of low oxygen were followed. In figures 3E and 3F the effects of administration of low oxygen are shown in the femoral artery, in the internal carotid, common carotid and external carotid. Of the carotid flows the augmentation is greatest in the internal carotid, next greatest in the common carotid and absent in the external carotid.[2] It would seem from these records that the purer the cerebral flow the greater the augmenting effects of low oxygen.

The simplicity of the carotid curves of flow as compared with the femoral

[2] The post-administration drop noted particularly in the flow through the external carotid artery is not an infrequent finding (see figs. 3B and 3C). It is possible that this may explain in part the deleterious effects of low alveolar oxygen so commonly noted in these experiments.

curves is worthy of note. The absence of the extra oscillations is indicative of a small influence of the external carotid flow. There is, of course, the other possibility that in general the head flow varies as the brain flow. This would not be contrary to adequate protection of the highly organized sense organs of the head on which smooth function of the brain is dependent.

SUMMARY AND CONCLUSIONS

The effects of variations in alveolar oxygen pressure and of the intravenous injection of sodium cyanide on the carotid and femoral flow of blood were studied with the continuous electrometric method.

The administration of a gaseous mixture low in oxygen increased the carotid flow of blood and readministration of room air diminished the flow. Low alveolar oxygen produced an initial decrease in femoral flow of blood which varied in degree and duration. With prolonged administration the decreased flow gave way to increased flow above the pre-administration level. Re-administration of room air elicited a second rapid and transitory increased flow which gradually gave way to recovery. These changes in flow are not dependent on changes in driving head of pressure.

Sodium cyanide elicited in the main an increased carotid flow of blood. The effects of cyanide on the femoral flow of blood were more inconstant. Though eventually the flow was always increased, this increased flow was preceded by a decreased flow varying in degree and duration.

The administration of 50 and 100 per cent oxygen mixtures decreased the carotid and femoral flows and re-administration of room air increased the flows. In the femoral artery the decreased flow of blood produced by high alveolar oxygen was frequently preceded by a temporary increased flow.

Administration of low oxygen and of carbon dioxide elicited the same directional changes in the flow of blood through the vertebral artery as in the common carotid. It is, therefore, concluded that changes in flow of blood through the common carotid artery are indicative of changes in flow through the brain.

Reasons are also given for believing that the head flow varies in general as the brain flow.

A mechanism explaining the difference in flow through the brain and through the leg is suggested. The possibility of a central and peripheral acid mechanism of volume-flow control modified by the paralytic action of reduced oxidation is proposed.

The results are not in disagreement with the view that the metabolism of the vasomotor center may play a part in the control of volume-flow of blood.

BIBLIOGRAPHY

BRONK, D. W. AND R. GESELL. 1927. This Journal, lxxxii, 170.
GESELL, R. 1923. This Journal, Proc. Amer. Physiol. Soc., lxviii.
GESELL, R. AND A. B. HERTZMAN. 1926. This Journal, lxxviii, 206, 610.
TIVEL. 1927. Compt. rend. Soc. Biol. March. Reference: HALDANE. 1927. Physiol. Reviews, vii, 363.

STUDIES ON THE VISCERAL NERVOUS SYSTEM

Vasomotor Reflexes from the Abdominal Viscera

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Received for publication October 11, 1927

Carlson (1) noted changes in the heart rate and blood pressure during strong hunger contractions in man. Carlson and Luckhardt (2) found no such changes in the turtle. They did find in the turtle that strong distention of the stomach, mechanical or electrical stimulation of the gut, electrical stimulation of the urinary bladder, or electrical stimulation of the central ends of the gastric vagi or sympathetic trunks, produced a rise in blood pressure. This was usually accompanied by an acceleration of the heart rate but occasionally it occurred without acceleration.

Methods. Dogs anesthetized with barbital-sodium were used. Blood pressure was recorded from the carotid artery by the usual method. Plethysmographs and oncometers of the Jackson type were used. Vasomotor changes in the nose were recorded by the Tatum (3) method; also by varying the method to the extent of using a purse-string suture to close the posterior nares. Different methods of stimulation resulted in different vasomotor results, therefore the methods will be mentioned with the results.

Results. Gentle stroking of the parietal peritoneum with the fingers or with a smooth instrument produced a sharp rise in blood pressure in some cases (fig. 1) and in other cases a sharp fall (fig. 2); occasionally a diphasic response resulted. The response usually followed a stimulation within 1 to 2 seconds. The blood pressure change amounted to 50 to 60 mm. Hg. These blood pressure changes were not due to change in the heart rate for the latter did not change. Similarly either pressor or depressor responses were obtained by any one of the following methods of stimulation: a, traction on the small intestine, the large intestine, the omentum, the urinary bladder, the gall bladder, the uterus or the Fallopian tubes; b, distention of any hollow viscus; squeezing the testes. The results from different types of stimulation and from the same type of stimulation applied to different organs gave various responses. In some dogs distention of the small intestine produced a pressor response, whereas traction produced a depressor response. In other dogs dis-

tention of the small intestine and traction both produced a depressor response. In still other dogs a pressor response resulted from either type of stimulation. Similarly, distention of the urinary bladder caused a pressor response in some dogs but a depressor response in others. Squeezing the testes usually produced a marked depressor response but in some dogs a pressor response resulted. Thus, each abdominal organ is supplied by both pressor and depressor fibers (or mechanisms). We were unable to determine the factors tending toward pressor or depressor responses.

In general: the parietal peritoneum gave a pressor response as frequently as a depressor; the distention of the intestines gave pressor responses, traction on the intestines or mesentery gave depressor responses; squeezing the testes gave depressor responses.

By chilling the animal with wet towels we were able to change pressor responses to depressor responses. If soon after the pressor-depressor reversal had taken place the animals were warmed, the pressor response was again obtainable. If the animals were cooled beyond this stage, the reversal was permanent and no amount of warming would make it possible to obtain the pressor response.

Recalling the pressor-depressor reversal Reid Hunt (4) obtained by cooling the sciatic nerve, we cooled a portion of the gut in order to observe whether the reversal in our experiments was due to a peripheral phenomenon. It was found that the pressor response gradually decreased and was finally abolished but that a depressor response did not occur. We concluded, in consequence, that the pressor-depressor reversal was not due to cooling the afferent nerves of the reflex.

The fact that chilling the animal caused a pressor-depressor reversal suggested that this mechanism might be related to "shock." It was found that during the pressor-depressor reversal the splanchnic organs continued to respond by vasoconstriction and that the blood pressure fall was due to an increase in the degree of peripheral vasodilatation. The mere chilling of the animal did not affect the blood pressure and the periods of stimulation necessary to obtain vasomotor reflexes were so brief that it is very unlikely that there was dehydration of the blood by the tissues. Thus as there is neither splanchnic stasis nor oligemia it is unlikely that the pressor-depressor response which we obtained is related to "shock."

The tonus of the vasomotor center is also subject to reflex influence from stimulation of the abdominal viscera. In some cases in which the blood pressure was maintained at a very constant level except for the respiratory variations, visceral stimulation inaugurated pronounced Traube-Hering waves of 15 to 30 mm. Hg amplitude which lasted from fifteen minutes to several hours (fig. 3).

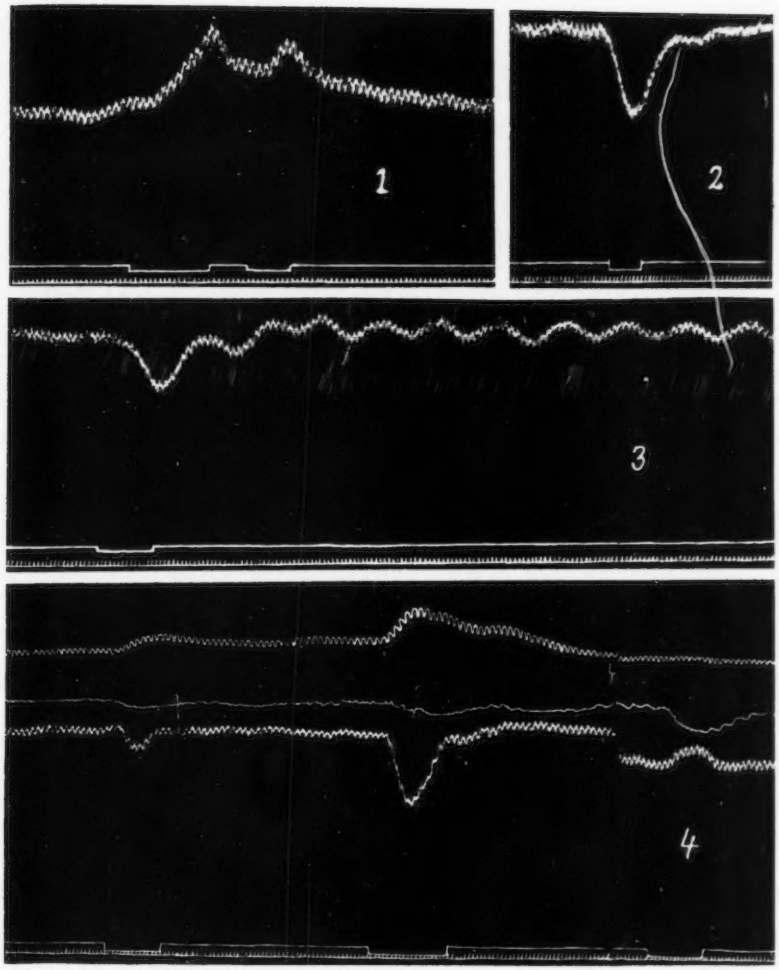

Fig. 1. Record of the blood pressure (in mm. Hg) of a barbitalized dog showing the marked pressor response resulting from stroking the parietal peritoneum with the finger. The signal indicates the period of stimulation. The signal line is the base line for the blood pressure. The time interval is 5 seconds.

Fig. 2. Legend same as in figure 1. The fall in blood pressure resulted from stimulation of the parietal peritoneum.

Fig. 3. Legend same as in figure 1. This tracing shows the Traube-Hering waves resulting from a brief stimulation of the intestines.

Fig. 4. Legend same as in figure 1, except for the upper two tracings. The top tracing is from a leg plethysmograph. The next to the top tracing is from an intestinal oncometer. The blood pressure variations are seen to be the resultant of these two. The first two stimulations were traction on the small intestine. The third stimulation was gentle distention of the small intestine.

In order to trace the redistribution of blood occasioned by our experimental procedures, we studied the blood volume changes of various organs resulting from stimulation of the abdominal viscera.

The small intestine invariably responded by vasoconstriction. Having previously ascertained (5) that stimulation of the gastro-intestinal tract produced inhibition of all parts of it except that part stimulated, it was necessary to rule out the possibility of mistaking relaxation of the gut for a vasoconstrictor effect. This was accomplished by placing a balloon inside of the segment of gut which was in the oncometer. We then observed that the pressure fell both in the oncometer and the inner balloon, that is, both inside and outside of the gut wall. This could have resulted only from decrease in blood volume, that is, vasoconstriction.

The kidney and spleen also responded by vasoconstriction. The mucosa of the nose and the frontal sinuses responded similarly. The hind leg responded by vasodilatation (fig. 4).

SUMMARY AND CONCLUSIONS

Adequate stimulation of the abdominal viscera reflexly influences both the reflex and the tonus activity of the vasomotor center.

Various stimuli applied to different organs produce in some dogs depressor responses and in other dogs pressor responses. The factors which determine whether the response shall be pressor or depressor have not been determined. The splanchnic area always responded by vasoconstriction and the limbs by vasodilatation. The blood pressure may be determined by the resultant of the two, the heart rate remaining constant.

By chilling the animal, a pressor reflex could be converted into a depressor one. This phenomenon was reversible if the chilling was not too prolonged, otherwise it was persistent. The pressor-depressor reversal is not due to cooling the afferent arc of the reflex as in the Reid Hunt sciatic experiment.

The pressor-depressor reversal is not related to "shock," because a, there was not time for a tissue dehydration of the blood; b, there was vasoconstriction of the splanchnic area and never vasodilatation; c, the heart rate remained unaltered; d, the blood pressure did not fall except during the period of visceral stimulation.

The tonus of the vasomotor center in barbitalized dogs maintains a very uniform blood pressure level. Visceral stimulation frequently produced large Traube-Hering waves which persisted a quarter of an hour to two hours.

We take pleasure in acknowledging our thanks to Professor Carlson for his constructive criticism in this work.

BIBLIOGRAPHY

(1) Carlson, A. J. This Journal, 1913, xxx, 318.
(2) Carlson, A. J. and A. B. Luckhardt. This Journal, 1921, lv, 31.
(3) Tatum, A. L. This Journal, 1923, lxv, 229.
(4) Hunt, R. Journ. Physiol., 1895, xviii, 387.
(5) Pearcy, J. F. and E. J. Van Liere. This Journal, 1926, lxxviii, 64.

THE NATURE OF THE FOODSTUFFS OXIDIZED TO PROVIDE ENERGY IN MUSCULAR EXERCISE

I. In the Normal Animal

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Received for publication October 4, 1927

Chauveau in 1896 published in brief form an experiment performed on Tissot. In this experiment the respiratory quotient rose from a resting-level of 0.75 to 0.95 after 45 minutes of running up and down stairs. Following the work, after one hour's rest, the R.Q. was 0.74. Chauveau formulated the theory that muscular exercise was accomplished at the expense of carbohydrate. This was immediately attacked by Zuntz (1896), who stated that all foodstuffs were equally capable of furnishing the energy for muscular work, without previously being changed to sugar. A long series of researches from his laboratory established in Zuntz's mind the validity of this conclusion, which he recapitulated in 1911.

About this time began the work, originating in the experiments of Fletcher and Hopkins, and carried out largely by A.V. Hill and Meyerhof and their collaborators, showing the importance of lactic acid in muscular contraction. This has resulted in a shift of opinion, with a tendency in many quarters to consider carbohydrate as the sole fuel for muscular exercise, and to disregard fat in this connection, save perhaps as a source of carbohydrate. Until recently, the reasons for this point of view have been mainly indirect and based chiefly upon observations made on excised muscle.

What positive evidence is there to lead one to the belief that in the organism as a whole, fat might be utilized to provide energy for muscular exercise? The experiments of the Zuntz school had shown that in man, as in other animals, the R.Q. of exercise was variable, and depended to a large extent on the diet, being higher on a high sugar and lower on a high fat diet. Anderson and Lusk (1917) found that in a fasting dog running 9 miles over a period of 3 hours, the R.Q. of exercise was almost the same as the basal R.Q., both approaching the theoretical value for fat. Krogh and Lindhard (1920), giving men "moderate work of constant intensity" (about

[1] Crile Research Fellow.

2 hours' riding on a bicycle ergometer with approximately a five-fold increase in O_2 consumption) observed that the R.Q. of exercise varied with the diet to which the individual had been subjected. They also showed a loss of efficiency when fat was being utilized and tentatively stated that this might be due to the conversion of fat to carbohydrate, with the subsequent combustion of the latter. Meyerhof and Himwich (1924) caused rats to run in a treadmill for approximately one hour, and observed that the exercise R.Q.'s approximated the basal R.Q.'s and corresponded to the previous diet of the animals. Henderson and Haggard (1925), studying severe work by trained athletes, also observed a correspondence between the resting R.Q. and that of exercise even when they indicated the combustion of a pabulum which was chiefly fat.

On the other hand, Benedict and Cathcart (1913) noted a tendency for a higher R.Q. during exercise than at rest (though this was not invariable). After exercise there was frequently a fall in the R.Q. to a point below the resting level. The necessity for measuring the total gaseous exchange of exercise and recovery, or of making certain that a "steady state" obtained at the time of the observations, was not fully recognized. The experiments were consequently inconclusive. Similar arguments apply to the work of Campbell, Douglas and Hobson (1921) and also to that of Chauveau. (While the neglect of the above mentioned precautions would also affect the validity of the exercise R.Q.'s obtained by workers whose results indicate a utilization of fat, it should be noted that if anything, the error would be in the direction of too high rather than too low quotients.) L. J. Henderson and his co-workers (1927) have published a note on exercise, during the last 20 minutes of which the R.Q. of the excess metabolism was 0.95, indicating an almost exclusive combustion of carbohydrate. No mention is made of the time after food ingestion that the exercise was performed, or of the nature of the diet. The basal R.Q.'s (0.91 to 0.96) make it appear that even in the resting state, carbohydrate was the preferential fuel for the organism.

But the most important evidence of the exclusive use of carbohydrate as the source of energy for muscular exercise has come from the laboratory of A. V. Hill. As early as 1924, Hill stated that "the primary fuel of muscle is carbohydrate", and that "it is difficult not to believe that the utilization of fat by muscles can occur only after its previous 'conversion' somewhere in the body". Subsequent work from his laboratory has tended to confirm at least the first portion of this opinion. Furusawa, Hill, Long and Lupton (1925) observed that the R.Q. of the excess metabolism of exercise and recovery was unity, even on a diet of fat and protein, provided the exercise was not too long. When the length of the exercise was increased, the excess R.Q. fell; in one case it was 0.71, in another 0.63. They concluded tentatively that in exercise of short duration, when the "general

metabolism of the body is not affected", the exercise is paid for primarily by the oxidation of carbohydrate. In a paper by Furusawa (1925) this conclusion is stated more positively Noting that the excess R.Q. of exercise may vary with the diet and that it sometimes does not rise appreciably, he states that this is only true in severe or long continued exercise. The experiments were performed on human beings. On a normal diet, the excess R.Q. of exercise was found to be practically unity even on 30 minutes of "standing running" at 120 steps per minute, the metabolism being increased about threefold. When the individual indulged for as much as 20 minutes in a more severe exercise—one involving an almost eightfold increase in the O_2 consumption— the excess R.Q. was still 0.98. On a high fat diet the R.Q. of the excess metabolism was also unity for short exercise. Even after 9 minutes of "standing running", when the O_2 consumption rose from 250 cc. to 1900 cc. per minute and "the subject felt as though he had walked 20 miles", the excess R.Q. was 0.95. When the exercise was more severe or prolonged, the R.Q. tended to fall.

The above results led to the conclusions that carbohydrate is the exclusive direct source of energy for muscular contraction and that fat is called upon only in exercise that is severe or of long duration and then only to supply additional carbohydrate. Presumably, experiments such as those of Anderson and Lusk, Krogh and Lindhard, or Henderson and Haggard, would be explained, on this hypothesis, by the duration or severity of the exercise.

As to the first of these conclusions, that carbohydrate is the primary fuel for muscular exercise, it is chiefly based on an R.Q. of unity in short mild exercise in a normal animal on any diet. This is a question of fact, with which the experiments that we are here reporting are in disagreement. As to the second conclusion, that fat is only utilized to replenish depleted carbohydrate stores, it is, we believe, as yet an unproved assumption, which certain of our facts appear to contradict.

Experimental procedure. Our experiments were made on four dogs who ran on a horizontal treadmill. The exercise was performed in the post-absorptive state (with one exception of which special mention will be made later), usually 18 to 20 hours after the last food. The animals were given three different diets. The "normal diet" was the maintenance diet, on which the animal customarily lived. It contained about 55 to 60 per cent carbohydrate, 25 to 30 per cent fat, and 15 per cent protein. The second, or high carbohydrate diet, consisted of about 90 per cent carbohydrate and 10 per cent protein. The third, or high fat diet, consisted of about 90 per cent fat and 10 per cent protein. In order to prevent excessive depletion of the body carbohydrate stores, the latter diet was only continued for two days prior to an experiment, as was the high carbohydrate diet, for purposes of comparison.

Each dog was subjected to a period of training before the actual experiments were undertaken, in order to accustom the animal to the apparatus and to make sure that she would lie quietly during the resting periods and run efficiently when the treadmill was moving. On three of the four dogs we established the approximate basal metabolism before beginning the exercise experiments. We did this so that we might know approximately what O_2 consumption we might expect with the animal at rest. We were unable, in the experiments involving exercise, to record the exact amount of movement occurring during the rest periods, because the usual

TABLE 1

Alcohol checks

DATE	EXPERIMENT NUMBER	CALORIES PER HOUR	R.Q.
1927			
February 28	22		0.667
March 9	23	23.83	0.667
March 30	24	22.64	0.653
April 20	25	34.64	0.669
April 23	27	12.73	0.679
April 28	29	12.84	0.665
May 1	31	12.01	0.660
May 2	32	11.17	0.658
May 4	34	13.68	0.659
May 5	35	14.78	0.666
May 8	36	15.24	0.671
May 12	37	13.51	0.664
May 16	38	13.61	0.660
May 21	39	10.82	0.667
May 26	40	14.05	0.654
May 27	41	14.91	0.663
Average			0.664

Mean R.Q. deviation ..... 0.003 (0.4 per cent error)
Maximum R.Q. deviation ..... 0.014 (2.1 per cent error)

recording device in the apparatus was, for the purposes of this research, replaced by the treadmill. However, by observation of the spirometer, which betrayed any unusual movement, and by a knowledge of the expected O_2 consumption, we were able to judge with a fair degree of accuracy whether the animal had been quiet during a given period. This was supplemented by occasional direct observation of the dog in the chamber.

The exercise in our earlier experiments consisted of 45 minutes' running at a speed of from 1.2 to 1.6 miles per hour. At this speed, the dogs showed no signs of fatigue. However, in our later experiments, we cut down the time of exercise to 15 minutes, with the animal running at the rate of 1.2

miles per hour. This is obviously a mild exercise, as mild as the animals would be apt to undergo under natural living conditions. It involved a threefold to fourfold increase in the O_2 consumption per minute. At a treadmill speed less than the one we adopted, the animals, as we discovered, would not have been able to run efficiently.

The apparatus used for determining the metabolism was a closed system of the Benedict-Homans type and has been described in previous communications from this laboratory. The CO_2 and water were collected in soda lime and sulphuric acid, respectively, and weighed directly. The O_2 was determined by noting the loss of weight in an O_2 tank by means of which the system was kept at constant volume, and making suitable corrections for changes in temperature, barometric pressure, and residual CO_2 and water.

The apparatus, as was shown by sixteen alcohol checks performed in the course of the research, was accurate to within approximately 2 per cent. These checks are summarized in table 1. The average respiratory quotient was 0.664, showing a mean deviation of 0.003, or 0.4 per cent from the theoretical value. On one occasion an R.Q. of 0.653 was obtained, on another, one of 0.679, showing a maximum deviation of 0.014, or 2.1 per cent. In 13 of the 16 experiments, the error in the R.Q. was no greater than 0.007. In the first two alcohol checks, on February 28th and March 9th, the treadmill was alternately started and stopped, as in the conditions of the animal experiments. The R.Q.'s indicated that the accuracy of the apparatus was not impaired under these circumstances. It will be manifest from the table that wide variations in the heat production also did not affect the accuracy of the system.

The horizontal treadmill consisted of an endless leather belt on rollers which were turned by a shaft propelled by a $\frac{1}{2}$ horse-power motor. The shaft passed through the wall of the animal chamber and through the water jacket surrounding the chamber. Leakage of water or air into the chamber was prevented by stuffing-boxes and by an oil bath on the outside wall of the water jacket.

The routine procedure of the experiments was as follows. A resting metabolism was obtained, first allowing an hour period for equilibration of the system. The resting periods usually consisted of two half-hour periods, though more were obtained if the animal showed evidence of restlessness or if there was appreciable variation in the R.Q. This was followed by the exercise. In the case of both the 45 minute and the 15 minute exercises, 15 minutes more, during which the animal rested, were allowed before the period was closed. This was done in order that the temperature and the residual CO_2 and water in the apparatus might more nearly reach an equilibrium. When these factors are changing rapidly, the corrections for them are not always accurate. This is particularly true in the case of

the residual water, which may introduce an appreciable error. The evidence indicated that in the 15 minute exercise, all of the O_2 debt had been paid by the time the 15 additional minutes of rest had passed and the experimental period was closed. Nevertheless, even at the end of this time, the assumption that this period represented the actual metabolism of exercise and recovery would, in some cases, have involved an error, usually in the direction of too high a quotient. Apart from this possible technical source of error involving the oxygen, the R.Q. of the period following the exercise period was almost invariably lower than that of the final resting quotient. This was true even when the O_2 intake had approximated basal levels, and doubtless represented CO_2 retention by the blood. We have therefore always included the period following that of exercise in our calculation of the metabolism of exercise and recovery. Since we were not interested in either the exercise metabolism or the recovery metabolism separately, but in the total of both, this procedure was justified. In no case did recovery extend beyond this second half-hour period. Following this, additional half hour periods were run with the animal at rest until we were certain that basal conditions again obtained. Usually, the exercise period and the additional half-hour period were followed by at least two more periods, occasionally more when there was any appreciable fluctuation in the R.Q., or when the animal betrayed restlessness. In two experiments the post-exercise basal metabolism could not be obtained, as the animal was quite restless. On a few other occasions the dogs were not equally quiet during the basal periods before and after exercise, though the differences in heat production did not exceed 10 per cent of the basal.

The excess metabolism was obtained as a routine by deducting the average CO_2 and O_2 of the pre-exercise and post-exercise basal periods from those of the exercise and recovery periods, calculated on a basis of one hour.

The animals were catheterized before and after the experiments and the urinary nitrogen determined, permitting calculation of the non-protein metabolism.

EXPERIMENTAL RESULTS. *a. The resting non-protein respiratory quotients before exercise and after recovery.* Before going on to the question of the R.Q. of the excess metabolism of exercise, it will be advisable to discuss the quotients of the pre and post-exercise basal periods. Hill, Long and Lupton (1924) state that following exercise the resting R.Q.'s are consistently somewhat lower than that of the resting metabolism before exercise. While the average of the post-exercise basal in our experiments is slightly lower than that of the pre-exercise basal, the difference is within the limit of error (table 2). It will be seen, moreover, that the variation is not consistent. In 8 of the experiments the R.Q.'s were the same, in 6 lower, and in 2 higher. In one experiment, after a high carbohydrate diet, the R.Q. dropped from a pre-exercise value of 1.015 to 0.934 after the exercise. It would appear

that during the exercise sufficient carbohydrate was used up to alter the character of the basal metabolism. As a rule, however, the differences were slight. If any change in the R.Q. following recovery from exercise was due to the exercise itself, it would probably be more logical to base the calculation of the excess metabolism upon the difference between the metabolism of exercise and that of the resting period before exercise. This would have tended very slightly to lower the R.Q. of the excess due to exercise. If, on the other hand, the variations in the two basals were due to continuation of the fasting state, or to any other cause, it would be better to use the average of the two resting values as a basis for comparison. This is the course we have followed. Using either method, the validity of our conclusions is unimpaired.

TABLE 2

Comparison of the non-protein R.Q.'s of the resting metabolism before and after exercise

	LOWER AFTER EXERCISE	THE SAME*	HIGHER AFTER EXERCISE
	Number of experiments		
"Normal" diet	2	2	1
High carbohydrate diet	2	3	0
High fat diet	2	3	1
Total	6	8	2

Maximum deviations in R.Q.	Lower	0.05†
	Higher	0.03
Average R.Q. of pre-exercise basal		0.842
Average R.Q. of post-exercise basal		0.832

* Within the limit (0.01) of experimental error.

† Excluding one of 0.08 in which the R.Q. before exercise was 1.015, and after exercise dropped to 0.934. Discussed in text.

b. Experiments involving 45 minutes' running at 1.2 to 1.6 miles per hour. The earlier experiments, involving 45 minutes' exercise on the treadmill, are summarized in table 3. They were performed on two dogs. The first of these, dog 4, weighed about 7.5 kilos and ran at the rate of 1.6 miles per hour, accomplishing approximately 15,000 kgm. of work. On the "normal" diet, containing 25 per cent fat and 60 per cent carbohydrate, the average non-protein respiratory quotient of the resting periods in one experiment was 0.844. The quotient of the exercise and recovery periods was 0.862. From these the R.Q. of the excess metabolism could be calculated as 0.891. (We have disregarded changes in the protein metabolism during exercise, and have assumed that the excess R.Q. is due entirely to carbohydrate and fat, which involves a negligible error. On this basis, the non-protein R.Q., rather than the total R.Q. of the resting periods, is the more valid standard

TABLE 3

Data of experiments involving 45 minutes' exercise on treadmill at 1.2 to 1.6 miles per hour

DOG NUMBER	EXPERIMENT NUMBER	DATE	DIET*						URINARY NITROGEN	BASAL (BEFORE EXERCISE)		BASAL (AFTER EXERCISE)		AVERAGE NON-PROTEIN R.Q. OF BASALS BEFORE AND AFTER	TOTAL R.Q. OF EXERCISE AND RECOVERY	EXCESS DUE TO EXERCISE	
			M.	C.M.	L.	Cals.	Fat	Carb.		O_2	Non-protein R.Q.	O_2	Non-protein R.Q.			O_2	R.Q.
		1927	*grams*	*grams*	*grams*		*per cent*	*per cent*	*grams per hour*	*grams per hour*		*grams per hour*				*grams*	
4 Weight 7.5 kilos	7	March 10	75	75	15	510	25	60	0.091	3.31	0.831	3.14	0.857	0.844	0.862	4.67	0.891
	8	March 12	50		50	500	90	†	0.099	3.58	0.750	3.51	0.715	0.733	0.742	5.99	0.744
	9	March 14	75	75	15	510	25	60†	0.095	3.18	0.880	§	§		0.884	5.33	0.911
7 Weight 9 kilos	3	March 26	80	80	20	580	30	55	0.114‡	5.34	0.849	¶	¶		0.849	8.35	0.859
	4	March 28	60		55	555	90	†	0.114	5.00	0.724	5.60	0.721	0.722	0.743	10.28	0.746
	5	March 31	60	130		580		90†	0.114	4.68	0.933	5.16	0.939	0.935	0.903	10.11	0.896

* M. = Lean beef heart; C. M. = Cracker meal; L. = Lard. All experiments were in the post-absorptive state—18–20 hours after food.

† For two days preceding the experiment.

‡ Dog usually voided before catheterization after the experiment. N. was taken as an average.

§ Not obtained; animal restless.

¶ Not obtained.

for comparison with the excess R.Q.) In another experiment on a normal diet this dog showed a non-protein quotient in the resting periods of 0.880 and an excess quotient of 0.911. Averaging these two experiments, it appears that during exercise and recovery, 66 per cent of the total non-protein metabolism was carbohydrate, as compared with 52 per cent during the resting periods. The difference is not striking.

When the animal had been fed a diet containing 90 per cent fat for two days preceding the experiment, the basal non-protein R.Q. was 0.733; the excess R.Q., 0.744.[2] In both cases it was evident that fat was being burned almost exclusively.

In the case of dog 7, who weighed 9 kilos and was a much fatter animal than dog 4, the correspondence between the resting and exercise metabolism is marked. On a "normal" diet, the basal non-protein R.Q. was 0.849; the excess R.Q., 0.859. On a high fat diet, there was an almost equal fall in both the resting and the excess quotients, the former being 0.722, the latter 0.746. When the dog had been on a diet containing 90 per cent carbohydrate for two days, the R.Q. of the resting metabolism was 0.933 that of the excess metabolism was 0.896, the quotient of exercise showing even less tendency to approximate unity than was apparent during rest.

The exercise in these experiments was not severe, but it might be criticized on account of its duration. In our later experiments, therefore, we shortened the period of running.

c. Experiments involving 15 minutes' running at 1.2 miles per hour. The experiments are summarized in table 4. They were performed on three dogs. Dog 6, weighing 6.5 kilos and performing about 3,300 kgm. of work, had, on a "normal" diet, the high resting quotient of 0.929. On exercise this was practically unaltered, the excess R.Q. being 0.932. On a high fat diet, the basal quotient was 0.745, while the excess quotient was 0.768, the one representing the utilization of a pabulum of which 13 per cent was carbohydrate, the other a mixture containing about 20 per cent carbohydrate, an insignificant difference. On a high carbohydrate diet, the non-protein R.Q. of the resting metabolism was above unity both before and after the exercise (1.051 and 1.031). The calculated R.Q. of the excess metabolism was 1.061. The actual quotient of the exercise and recovery periods, however, was 1.008, and it is possible that conversion of carbohydrate into fat, occurring during rest, may have been suspended during exercise, when the demand for fuel was increased.

Dog 8, on a "normal" diet, showed identical resting and excess R.Q's, the

[2] In a preliminary report (1927) we mentioned one excess R.Q. of 0.72. It was to this quotient that we had reference. A re-checking of the calculations showed that it should have been 0.74. We wish to take the opportunity to correct this mistake. Similarly, the highest excess R.Q. on the "normal" diet should have been 0.91.

TABLE 4

Data of experiments involving 15 minutes' exercise on treadmill at 1.2 miles per hour

DOG NUMBER	EXPERIMENT NUMBER	DATE	DIET*						URINARY NITROGEN	BASAL (BEFORE EXERCISE)		BASAL (AFTER EXERCISE)		AVERAGE NON-PROTEIN R.Q. OF BASALS BEFORE AND AFTER	TOTAL R.Q. OF EXERCISE AND RECOVERY	EXCESS DUE TO EXERCISE	
			M.	C.M.	L.	Cals.	Fat	Carb.		O_2	Non-protein R.Q.	O_2	Non-protein R.Q.			O_2	R.Q.
		1927	*grams*	*grams*	*grams*		*per cent*	*per cent*	*grams per hour*	*grams per hour*		*grams per hour*				*grams*	
6 Weight 6.5 kilos	5	May 6	75	75	15	510	25	66	0.089	3.00	0.947	3.18	0.895	0.929	0.910	1.46	0.952
	6	May 10	51		51	510	90	†	0.092	3.32	0.753	3.58	0.738	0.745	0.766	1.25	0.768
	7	May 13	60	115		520		80†	0.086	3.36	1.051	3.29	1.031	1.041	1.008	1.18	1.06†
7 Weight 9 kilos	9	May 5	60		58	580	90	†	0.114	3.94	0.744	3.86	0.745	0.745	0.772	2.27	0.782
	10	May 7	75	125		575		85†	0.114	3.62	1.014	3.58	1.005	1.011	0.963	2.27	0.967
	11	May 11	80	80	20	580	30	55	0.114	4.00	0.945	3.94	0.901	0.923	0.868	2.03	0.817
	13	May 24	80	80	20	580	30	55‡	0.114	4.36	0.806	4.31	0.816	0.811	0.807	2.12	0.799
8 Weight 9 kilos	1	May 14	80	80	20	580	30	55	0.111	4.67	0.855	4.08	0.865	0.859	0.852	2.80	0.858
	2	May 18	60		58	580	90	†	0.101	4.24	0.742	4.06	0.743	0.743	0.734	2.95	0.703
	3	May 20	75	125		575		87†	0.097	4.08	1.015	4.24	0.934	0.973	0.966	2.48	1.049
	4	May 25	75	40	40	595	60	27†	0.105	4.00	0.809	3.86	0.826	0.818	0.823	3.08	0.836
	5	June 2	75	125		575		87†	0.100	4.54	1.021	4.04	1.026	1.023	0.985	3.02	0.992

* All experiments except experiment 13 on dog 7 were performed in the post-absorptive state.

† For two days preceding the experiment.

‡ In this experiment 75 grams lard were given to the animal 2 hours before exercise.

former being 0.859, the latter 0.858. On a diet containing 90 per cent fat, this animal's resting R.Q. was 0.743. The excess metabolism in this instance had a respiratory quotient of 0.703, or practically the theoretical value for fat. We regard this as significant, although the almost exact coincidence is no doubt accidental.

This dog was also given a diet containing 60 per cent fat and 27 per cent carbohydrate. On this relatively high fat diet the basal quotient was 0.818, and the excess quotient 0.836.

Two experiments were performed with this animal on a high carbohydrate diet. We have already alluded to the basal non-protein quotients of this experiment. Before exercise the quotient was 1.015; after exercise it was 0.934. With a rapidly changing R.Q. such as this, which we validated at once with an alcohol check, and the authenticity of which we have no reason to doubt, it is difficult to calculate the excess R.Q. with exactitude. The actual R.Q. of the exercise and recovery periods was 0.966; and that of the excess metabolism, calculated upon the average of the basal, 1.049. Probably the true excess R.Q. was approximately unity. On repeating this experiment, the resting non-protein quotients were more consistent, and were above unity. The excess quotient was 0.922. In this case it seems evident that the conversion of carbohydrate to fat was abolished during the exercise.

Dog 7 had been subjected to the 45 minute exercise. When she was caused to run 15 minutes on a "normal" diet, the basal R.Q. was 0.923. The experiment introduced the only marked deviation that we observed from the general agreement of the resting and excess quotients, for in this case the excess R.Q. was 0.817. We have no reason to question the validity of the data in this experiment.

On a diet rich in carbohydrate the resting non-protein quotient was 1.011, or practically unity. The quotient of the excess metabolism was 0.967. On a high fat diet the excess R.Q. was somewhat higher than the resting value, being 0.782 as compared with 0.745.

It was clear from this and other experiments that when an animal was on a high fat diet, the respiratory quotient of the excess metabolism was low, in one case approximating the theoretical value for the combustion of fat. With such experiments alone in evidence, it might be argued that the preferential combustion of fat (or, on another conception, the conversion of fat to carbohydrate) was due to the fact that the available carbohydrate stores had been depleted as a result of the two days on a fat rich diet. In spite of the fact that the caloric needs of the animal were satisfied by this diet, such an argument might be advanced if it were held that the muscular exercise of these two days was solely at the expense of carbohydrate. Against this conception, however, we have the evidence of the experiments in which the animal was on a "normal" diet, containing 55 to 60 per cent carbohydrate, and in which the excess R.Q. was certainly not unity.

We have, moreover, performed the following experiment. We gave dog 7, then subsisting on a "normal" diet, 75 grams of lard 2 hours before exercise. The basal quotient, which had been 0.92 on the "normal" diet, was now 0.811. The quotient of the exercise and recovery periods was 0.807, as opposed to 0.868 on the "normal" diet with this exercise, and was considerably lower than the excess R.Q. (0.859) of this dog performing the 45 minute exercise. We therefore regard it as confirming the view that low quotients of the excess metabolism of exercise are not the result of the fact that there is practically no carbohydrate left for oxidation.

These results are briefly summarized in table 5, from which it is clear that the percentage of carbohydrate oxidized in the excess metabolism differs

TABLE 5

Comparison of the percentage of carbohydrate oxidized in the resting metabolism and in the excess metabolism of exercise

		RESTING METABOLISM		EXCESS METABOLISM OF EXERCISE	
		R.Q.	Carbohydrate per cent	R.Q.	Carbohydrate per cent
45-minute exercise	"Normal" diet	0.858	51.5	0.887	61.2
	High carbohydrate diet	0.884	60.4	0.911	69.6
	High fat diet	0.728	7.2	0.745	13.0
	Average	0.823	39.6	0.848	47.8
15-minute exercise	"Normal" diet	0.904	67.3	0.869	55.3
	High carbohydrate diet	1.012	100.0	1.017	100.0
	High fat diet	0.744	12.7	0.751	15.0
	Average*	0.860	52.2	0.854	50.2
Average of both exercises		0.846	47.5	0.852	49.5

* Includes experiment on a "moderately high" fat diet, and experiment in which fat was given two hours before exercise.

very little from that oxidized in the resting metabolism. Such differences as exist cannot be regarded as significant. In only one instance was the deviation marked, and this was in experiment 11 of dog 7, already alluded to. In this case 73.7 per cent of the non-protein metabolism was due to carbohydrate in the resting metabolism, and only 37.6 per cent in the excess metabolism. This sole exception can hardly affect the validity of the general rule, and certainly would not indicate that carbohydrate is the only fuel of exercise. The correspondence was as marked in the shorter as in the longer period of running.

Discussion. It is generally admitted that in severe or long continued exercise, the respiratory quotient of the excess metabolism of muscular

exercise is not necessarily unity. The point of view advanced by Furusawa, Hill, Long and Lupton (1925) and by Furusawa (1925), that carbohydrate alone supplies the energy for muscular contraction, is, as we have stated, based, apart from the experiments on isolated muscle, on an excess quotient of unity in mild, short-continued exercise. The evidence that we have adduced in this communication indicates, however, that in such an exercise the respiratory quotient of the excess metabolism tends to approximate the quotient at rest, and that both are functions of the diet to which the animal has been subjected. The exercises performed in our experiments may justifiably be compared with those performed in the experiments reported by the above named authors. An approximation to unity has only been obtained under conditions when there was a carbohydrate plethora, and not necessarily even then, if the resting non-protein R.Q. was less than one.

The evidence obtained from isolated muscle to indicate that the energy for muscular contraction is derived solely from carbohydrate is not proof that this is what occurs in the intact organism. It has been shown (Leathes, 1906; Winfield, 1914) that stimulation of muscles leads to no change in their fat content. But Lafon (1913) has shown that more fat was removed from the blood flowing to a muscle when the latter was at work than when it was at rest. It may well be that before fat can be utilized in muscular contraction it must first be changed into available form elsewhere in the body.

It is advanced that the function of the fat, when its use is indicated, is to replenish depleted stores of carbohydrate, which is then oxidized to supply energy for both exercise and recovery. It might be argued that experiments showing the R.Q. of excised stimulated muscle to be unity indicate that in the intact animal, when fat is used, it is first changed to carbohydrate in other than muscular tissue, for example, the liver, to which this function is ascribed. Porges (1910) found that after cutting the liver out of the circulation the R.Q. rose from fat levels to those of carbohydrate (0.88 to 1.0); Burn and Dale (1924), using eviscerated cats in which the liver vascular system was excluded, obtained R.Q's approximating unity (0.99 to 1.06). But the liver has other possible functions than the hypothetical one of converting fat to sugar, among which is the rendering available, by desaturation or otherwise, of the fatty acids for oxidation. Space forbids adequate discussion of the subject, which is reviewed by Leathes and Raper (1925; vide especially chapter VIII), but it suffices to say that absence of evidence of fat oxidation in isolated muscles or in animals with the liver removed may be due to the loss of ability to oxidize fats, rather than to a lack of the mechanism for converting fat to carbohydrate.

The arguments against the conversion of fat to carbohydrate have been presented many times, and in this connection notably by Lusk (1925), who states that "it seems difficult to accept the explanation that fat must be

changed to lactic acid in order to provide energy for the oxidative recovery in muscle". Our evidence is in agreement with this point of view. The close approximation of resting and exercise quotients, whether both be high or low, would, on the theory that fat is converted to carbohydrate in order to supply the fuel for muscular exercise, either involve the long arm of coincidence or else necessitate the assumption that a similar process was going on in the resting state. The fact that on high fat diets the R.Q's of both the resting and the exercise metabolism tend to approach the theoretical values for the combustion of fat would involve the advocates of this theory in similar difficulties. When we obtained an excess quotient of 0.703, it seems laboring the argument to suppose that there was a conversion of fat to carbohydrate with the subsequent immediate oxidation of this carbohydrate, the total reaction simulating, in the respiratory quotient, the direct burning of the fat. Unless definite proof is furnished, more convincing than previous questionable evidence on this point, that in rest as well as in exercise fat must be changed to carbohydrate before being able to furnish energy, it would appear simpler to assume that under these circumstances fat is being directly oxidized.

The argument (Furusawa, 1925) that quotients approximating the theoretical value for fat may be as indicative of the conversion of fat to carbohydrate as of the actual combustion of fat, appears to us to throw equal doubt upon R.Q's in the neighborhood of unity. We have observed resting R.Q's above one associated with excess R.Q's that approximated unity. In these experiments, as well as in the work of other investigators, the excess quotient of unity is taken to mean that carbohydrate is being exclusively oxidized. If it be assumed that low quotients approximating those of the oxidation of fat may be due to the conversion of fat to carbohydrate, plus the oxidation of this carbohydrate, why is it not equally logical to postulate that quotients approximating unity are due to the conversion of carbohydrate to fat plus the oxidation of this fat? Such a conception is not untenable, but we believe the burden of proof to be upon its proponents.

Our experiments of course throw no light upon the question as to whether fat may furnish energy in the anaerobic phase of muscular contraction. Admittedly, the weight of evidence is in favor of the conversion of glycogen to lactic acid as the fundamental reaction of the initial phase, although the thermodynamic data do not entirely preclude the use of fatty acids. Conceivably the latter might act as lactic acid does. The more likely occurrence, however, is that the oxidation of the fatty acids may supply the energy for oxidative recovery. Lusk (1925) suggests that "with the help of β-oxidation, one might imagine 'micro-explosions' of acetic acid, which are able to furnish this energy". The change—lactic acid to glocogen —cannot be at the expense of the oxidation of lactic acid directly, if it be true that lactic acid, to be oxidized, must first be changed to a hexose form,

in itself an endothermic process. The energy for oxidative recovery may come from the oxidation of hexose or of fatty acids or of both.

Our general conclusion is that muscles behave, in respect to the type of fuel that they burn, in a manner not essentially dissimilar to the tissues of the body as a whole. They oxidize either carbohydrate or fat or both, depending upon the proportions in which these substances are presented to them in available form.

SUMMARY

1. Experiments are reported in which dogs were caused to undergo mild exercise of usually short duration on a horizontal treadmill, involving a three-fold to four-fold increase in the O_2 consumption per minute. The experiments were performed, with one exception, in the post-absorptive state, and on three types of diet: "normal", high fat, and high carbohydrate. The gaseous exchange was determined during exercise and recovery until the latter was complete, and in rest periods before and after.

2. The respiratory quotients of the excess metabolism of exercise and recovery corresponded closely to the resting quotients. On a 45 minute exercise and on a 15 minute exercise the percentages of carbohydrate oxidized in the excess metabolism were, respectively: 1, on a "normal" diet, 61 and 55 per cent; 2, on a high carbohydrate diet, 70 and 100 per cent; 3, on a high fat diet, 13 and 15 per cent. In one experiment on the latter diet the excess quotient was 0.703, or practically the theoretical value for the combustion of fat.

3. The evidence indicates that even in mild exercise of short duration carbohydrate is not the exclusive source of energy for muscular contraction; and that fat is utilized not to replenish depleted carbohydrate stores, but by its oxidation to supply energy for muscular exercise.

4. The muscles, like the tissues of the body as a whole, oxidize carbohydrate, or fat, or both (usually the last), the percentage of each depending upon the proportions in which these substances are presented to them in available form.

BIBLIOGRAPHY

ANDERSON, R. J. AND G. LUSK. 1917. Journ. Biol. Chem., xxxii, 421.

BENEDICT, F. G. AND E. P. CATHCART. 1913. Publ. Carn. Inst. Wash., clxxxvii.

CAMPBELL, J. M. H., C. G. DOUGLAS AND F. G. HOBSON. 1921. Phil. Trans. Roy. Soc., B ccx, 1.

CHAUVEAU, M. 1896. Compt. Rend. Acad. Sci., cxxii, 1163.

HENDERSON, L. J., D. B. DILL, C. VAN CAULAERT, A. FOLLING AND F. C. COOLIDGE. 1927. Journ. Biol. Chem., xxxvi, 79.

FURUSAWA, K., A. V. HILL, C. N. H. LONG, AND H. LUPTON. 1926. Proc. Roy. Soc., B xcix, 167.

HILL, A. V. 1924. Science, N. S., lx, 505.

HILL, A. V., C. N. H. LONG AND H. LUPTON. 1924. Proc. Roy. Soc., B xcvi, 455.

KROGH, A. AND J. A. LINDHARD. 1920. Biochem. Journ., xiv, 290.
LAFON, G. 1913. Compt. Rend. Soc. Biol., clvi, 1248.
LEATHES, J. B. 1906. Problems in animal metabolism. London.
LEATHES, J. B. AND H. S. RAPER. 1925. The fats. London.
LUSK, G. 1925. Biochem. Zeitschr., clvi, 334.
MEYERHOF, O. AND H. E. HIMWICH. 1924. Pflüger's Arch., ccv, 415.
PORGES, O. 1910. Biochem. Zeitschr., xxvii, 131.
RAPPORT, D. AND E. P. RALLI. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 964.
WINFIELD, G. 1914–15. Journ. Physiol., xlix, 171.
ZUNTZ, N. 1896. Arch. f. Physiol., 538.
1911. Oppenheimer's Handb. d. Biochem., Jena.

A QUANTITATIVE AND DESCRIPTIVE STUDY OF THE ELECTRIC RESPONSE TO ILLUMINATION OF THE ARTHROPOD EYE[1,2]

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Received for publication October 7, 1927

In addition to the fact that the electric potentials developed in photoreceptor organs may give to visual physiology an objective method capable of quantitative treatment, it is not impossible that these potentials may play an important rôle in the mechanism of the transformation of stimulus energy into nervous impulse. Current conceptions of the nature of the nervous impulse suggest that a local circuit set up in the terminal sensory nerve fibre is the final step in the peripheral sensory process, the remainder of the process being merely the propagation of this electrical disturbance along the nerve. But what the process within the sense cell may be, is not as yet known. Whether electric potentials which can be recorded are actually produced by this process, and whether such potentials as are recorded are in any part responsible for the initation of the nervous impulse are questions which cannot be answered *a priori*. In any case the interpretation of the potentials recorded from visual organs must be undertaken with the greatest caution, if for no other reason than on account of the anatomical complexity of the receptor tissue.

The electric phenomena associated with stimulation of the retina by light have been studied, for the most part, in vertebrates; but for purposes of quantitative analysis of these phenomena the arthropod eye has been found to possess certain advantages. This paper presents a description of some of the types of response obtained from the arthropod eye, and a quantitative investigation of one phase of the relation between stimulating light and electric response.

The electric action potentials developed in visual end-organs, especially in the vertebrate retina, are too well known to require description here (cf. Chaffee et al., (1), (2)); it is sufficient to say that the potential between the two surfaces of the retina undergoes a typical change when the retina is illuminated; and that the action potential of the retina may be

[1] Done with the aid of grants under the Henry Strong Denison Memorial Fund.
[2] Granted the William H. Howell Award, June, 1927.

obtained upon illumination of the intact eye, when it manifests itself as a change in the potential between the cornea and the fundus of the eye ball. In a previous paper I described a method whereby this action potential may be recorded without removing the eye from the body of the animal (3). The potential between the cornea of the eye and any point on the animal's body is recorded; the changes obtained on illumination of the eye are found to be identical with those obtained from the excised intact eye, or from the excised retina. The advantage of this method is that it permits the use of the intact animal, in which the circulation to the retina is undisturbed. The slow death of the retina, with the accompanying changes in its electric response, which takes place in the excised preparation, is thus eliminated, or at least delayed. But the use of the intact preparation is subject to limitations; definite changes in the form and magnitude of the electric response may take place within the time required for quantitative studies. However, use of the arthropod compound eye enables one to obtain reproducible results for a period of twelve hours or more; it is largely for this reason that I have selected these forms for use in a quantitative investigation of the electric response of photoreceptor organs.

APPARATUS AND METHOD. 1. The source of light (fig. 1, S) is a 500-W Mazda projection lamp, type T-20. The filament of this lamp consists of six vertical coils of wire suspended in the same plane (fig. 1 a). A system of lenses, L_1, forms an image of this filament at F_1, after the rays have passed through 5 cm. of distilled water at B. At F_1 is a shutter, and slit diaphragm which can be adjusted to accommodate the image of all six coils of the filament or of any one. The rays of light are again brought to a focus by means of the lens system L_2, at the point F_2. At this point there is a holder, W, in which can be inserted Wratten optical filters of desired density, and slit diaphragms of various sizes to control the portion of the image to be used. The plate at F_2, which contains a slit just large enough to accommodate the image of the entire filament, is built into the framework of the optical system, and to it is soldered a metal tube containing appropriate diaphragms to cut out rays reflected from its sides. The tube ends in a metal truncated cone, into the small end of which is fitted a $\frac{1}{6}$ inch microscope objective. The lenses at the tip of this objective have been removed, and a truncated cone of glass fastened in their place (fig. 1 B). The larger end of this glass cone is fitted to the lens of the objective, the smaller end is just large enough to accommodate the very small image of F_2. The length of the cone is such that the third image of the filament, F_3, lies about 0.2 mm. away from the surface of the glass. The surface of the glass cone, the inside of the tube, and all parts of the framework are rendered dead black by painting with shellac-lamp-black mixture. The optical system is well shielded, so that only direct rays from the lamp can get to the foci F_2 and F_3. The entire apparatus is in a room in which the light is kept well subdued during experiments.

The shutter at F_2 consists of two elements, of which one is a rapidly moving frame which carries an opaque sheet of metal. This frame moves on the end of an electromagnetically driven lever, so that the light may be turned on or off by means of a double-throw switch. The edge of this shutter moves across the entire six elements of the image of the filament in 0.025 second. The second element of the shutter is a heavy pendulum carrying an opaque screen at its end. In the center of the screen is an

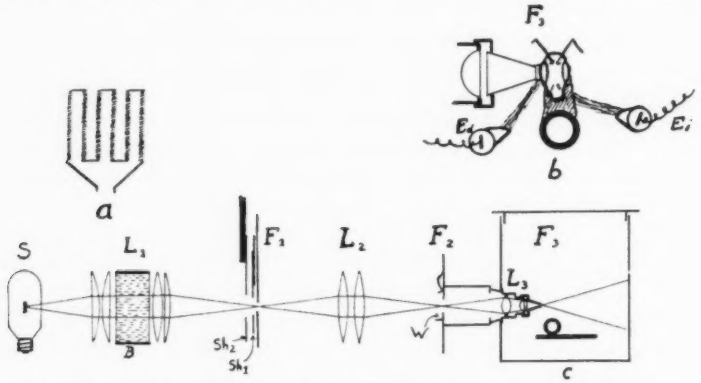

Fig. 1. Diagram of apparatus. S, source of light (500 W. Mazda Projection Lamp, type T20, burning 3.50 amps. at 118 volts); L_1, L_2, L_3, lenses; F_1, position of first image of S, formed by L_1; F_2, position of second image of S, formed by L_2; F_3, position of third image of S, formed by the modified microscope objective L_3. B, cell containing distilled water, to reduce heat from S. Sh_1, electro magnetic shutter for turning light at F_3 on or off; Sh_2, pendulum shutter for producing flash of light at F_3. W, position of Wratten "Neutral Tint" optical filters, for reducing intensity of light at F_3. C, dark-moist-chamber.

Insert a shows the pattern of the filament of S; its actual size is 1 cm. by 1 cm.; its image at F_3 is 0.1 cm. by 0.1 cm., and the illumination at F_3 is 85,000 meter candles, with no filters at W.

Insert b shows an insect mounted in the dark-moist-chamber, C, so that the image of S at F falls on the surface of the eye. A drop of saline solution serves as an immersion fluid at F_3, and permits electric contact to be made with the cornea of the eye, through the "different" electrode, E_d. The "indifferent" electrode, E_i, makes contact with the animal's body through the plaster of Paris mounting.

The figure is schematic, not drawn to scale.

opening of adjustable width, so located that when the pendulum is at rest the midde of this opening lies at the mid-point of the image at F_2, and permits free passage of the light rays. Two strong electomagnets, one on either side, can hold the pendulum displaced at a constant angle. A suitable double-throw switch will release the pendulum on one side and cause the magnet on the other side to catch it, producing a flash of light of the required duration by the passage of the slit through F_1. By means of

suitable interchangeable armatures carried by the pendulum, two angular displacements are available, so that two speeds of the slit can be obtained; one is 167 cm. per second, the other 47.8 cm. per second. By the use of slits varying in width from 0.63 to 130 mm., flashes of light from 0.00060 second to 0.28 second in duration can be obtained. When this pendulum is used, only one of the filament-coil images of the lamp is used; since the width of one of these is about 1 mm. the time required to pass from total darkness to full illumination and back again comprises, for all but the narrowest slits, a very small fraction of the total exposure time.

The Mazda lamp burns 3.50 amps. at 118 volts from a storage battery. The image at F_3 is 1 mm. square; the average illumination upon this area is 85,000 meter candles when no filters are used at F_2. The Wratten Neutral Tint Optical filters yield a range of intensities from 1 to 1×10^{-7}.

2. *The living preparation.* The microscope objective, bearing the glass cone and the image F_3, projects through the side of a small, light-tight, metal box containing the preparation. The animal is mounted on a paraffin-covered lead tube carried on an adjustable holder. This holder can be removed from the box, and when inserted can be adjusted so that the animal's compound eye just touches the tip of the glass cone, where it can then be clamped fast. The animal is fastened to the lead tube firmly enough to prevent free movement; care must however be taken not to cover the breathing organs or impede respiratory movements. As a rule this is done by means of stout threads about the animal's thorax and a small amount of bee's wax melted carefully about the base of the legs. The head is surrounded on one side by plaster of Paris, leaving one eye uncovered. Paraffin (M.P. 45°), containing lamp-black in suspension, is melted carefully over the exposed portion of the head, up to the margin of the eye. The chitinous exoskeleton of these animals, of which the cornea of the eye is a part, is thus held firmly by the plaster to the lead tube, and renders the eye immobile to an extent quite unobtainable in any vertebrate.

The adjustable holder, bearing the lead tube on which a preparation has been mounted in the manner described, is placed in the metal box; the animal's eye is brought up against the small end of the glass cone so that the curved surface of the cornea is tangent to the middle of the plane surface of the truncated cone. The image F_3 thus lies on the eye. A small drop of Ringer's solution is held by capillarity between the glass cone and the cornea; a fine cotton wick dips into this drop and leads to a cup of Ringer's solution fastened to the holder of the preparation. Another wick leads to a similar cup from some point on the animal's body—usually the plaster of Paris surrounding the head serves well for this purpose, if kept moist with Ringer's. The two cups containing Ringer's solution serve as the two electrical leads, each being connected by a saline bridge with respective calomel half-cells outside the metal box, which serve as non-

polarizable electrodes by means of which the preparation is connected with the galvanometer. A sketch, showing how the preparation is mounted with respect to the optical system and the electric recording system, is given in figure 1, insert *b*.

A stream of water at constant temperature runs through the lead tube and empties into the metal box, keeping the air in the box moist and at any desired temperature, within $\pm$ 0.1°C.

This technique permits the use of small arthropods, ranging in size from a crayfish to a house fly. Smallness of the eye, within moderate limits, is no drawback, since the electric contact is made through the immersion fluid which is part of the optical system. Electrode area remains constant throughout an experiment.

Most arthropods will survive at least 24 hours, and many show no change in the electric response to illumination of the eye during the course of 12 to 14 hours. A few individuals have survived for several days, yielding comparable electric responses over the entire period. Owing to the large electric resistance sometimes encountered in such preparations, it is often desirable to remove the coating of what is apparently a wax-like substance from the surface of the cornea. This may be done by gently scraping the surface of the cornea with the broken edge of a thin glass bubble.

3. *The electric responses* are measured by means of an Einthoven string galvanometer (3). Any difference in potential existing between the cornea of the eye and the point at which the "indifferent" electrode makes contact with the animal's body causes a current to flow through the galvanometer; even when the eye is unstimulated there may be a current of rest which indicates a difference of potential between the two points on the animal's body. This difference in potential is generally less than one millivolt; it may, however, become as large as five or ten millivolts, in either direction. When the eye is stimulated, there is a change in the potential between the two electrodes which causes the current flowing through the galvanometer to change, thereby deflecting the string. Since the resistance of the preparation remains constant over a long period of time, the string deflections, which are proportional to the current, may be reduced to potential difference by comparison with a suitable calibration potential impressed upon the circuit. The deflections of the string are recorded photographically in the usual manner, along with a time record and a signal marking the moment of turning the light on or off. Electric connections are made in such a way that when the cornea becomes negative with respect to the animal's body, the string is deflected upwards in the records.

Descriptive study. An electric response greater than 0.01 millivolt can be obtained only upon illumination of photosensitive tissue—i.e., of some visual end-organ (in animals). Illumination of pieces of muscle, connective tissue, integument of frog and of lobster, nerve fibre, nerve

ganglion (optic), and cornea has never effected any demonstrable change in the potential between the illuminated and dark surfaces. Indeed, some tissues known to contain photoreceptor end-organs—such as the skin of the anterior segments and prostomium of the earthworm, and the tip of the syphon of the clam *Mya arenaria*—also failed in my experiments,[3] to give any evidence of an electric response to illumination. It is only in the highly organized retinas of the eyes of higher animals that these responses can be demonstrated.

The simplest bioelectric response to illumination which I have observed occurs in the eye of the young *Limulus polyphemus* (2 to 8 cm. across the carapace). This response is reproduced in figure 2, *a*; it is a simple wave of potential, starting abruptly *ca.* 0.02 second to 0.03 second after the onset of illumination, reaching a maximum in *ca.* 0.5 second, and returning slowly toward its original value along a die-away curve, even though the light continues to shine steadily. On cessation of illumination there is a slight movement of the thread downward, toward the original base-line. The wave occurring at the onset of illumination is in such a direction that the cornea of the eye becomes more negative with respect to the back of the eye. *Limulus* is one of the few animals in which the excised eye might satisfactorily be used. It frequently survives for as long as 24 hours, giving reproducible responses over a period of 8 hours. Moreover, there is a long optic nerve, easily obtained, from which I have obtained very small but definite electric changes upon illumination of the eye.

The type of response found most frequently in arthropods is more complicated. In figure 2, *b*, is shown the form of action potential usually obtained. It consists of an "on effect" in response to the onset of illumination, and an "off effect" in response to the cessation of illumination. The "on effect" is a change in potential in such a direction that the cornea of the eye becomes more negative with respect to the back of the eye (through the external circuit). This "on effect" is composed of two parts — an "A" wave, and a "B" wave. The "A" wave is a rapid rise in potential, beginning after a latent period of 0.01 second to 0.02 second, reaching a maximum in about 0.08 second to 0.12 second, and then subsiding slightly. The recession of the "A" wave is interrupted by the second rise, the "B" wave. In a good preparation the "B" wave rapidly assumes a maximum value which it maintains as long as the intensity of the illumination is unchanged. The "off effect" is a simple decay of the "B" wave, beginning abruptly after a latent period of 0.08 second to 0.10 second. The base-line of potential before the onset of illumination is approached asymptotically, and the curve has a die-away form. It is sometimes preceded by a slight rapid wave in the direction of the "A"

[3] These experiments, and those on the eye of *Limulus*, were done at the Marine Biological Laboratory, at Woods Hole, Massachusetts, in the summer of 1926.

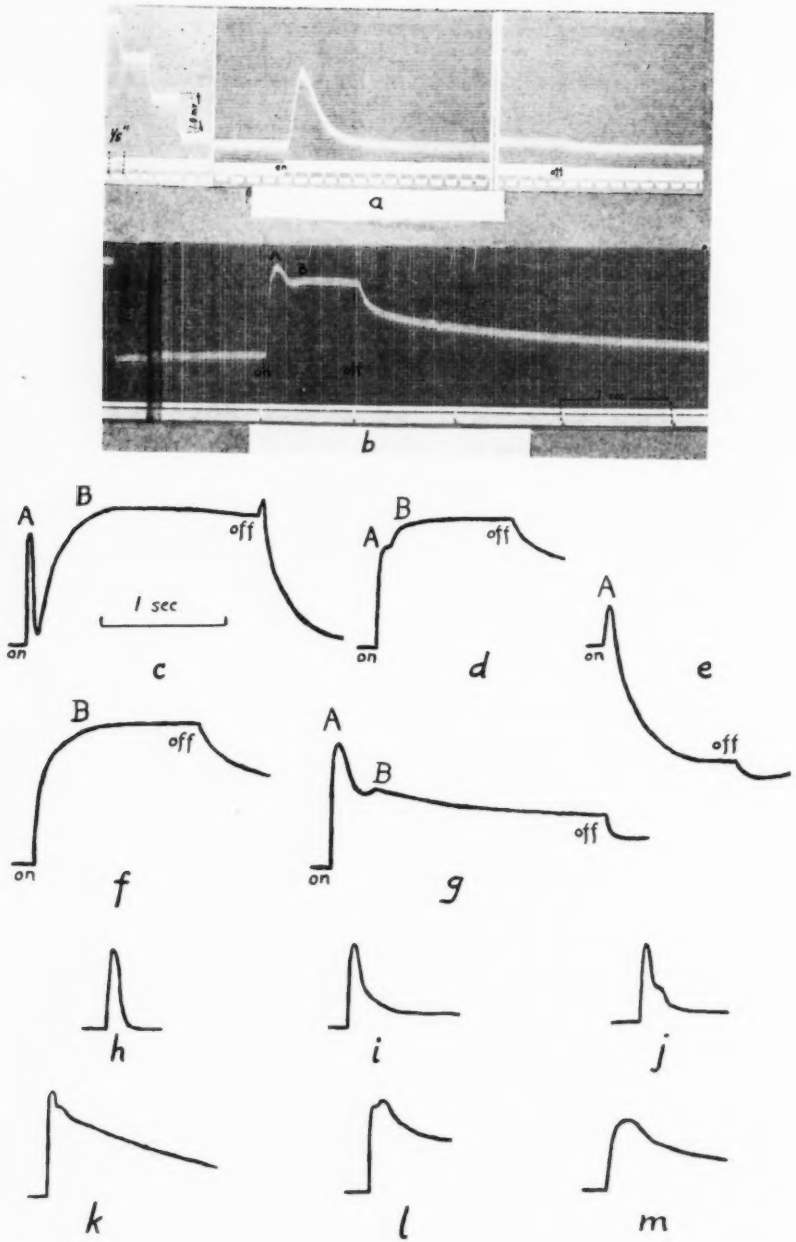
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Fig. 2

wave of the "on effect." This typical response is seen to best advantage in the grasshopper, (*Melanoplus femur-rubrum*) and in the bumble bee (*Bombus pennsylvanicus*). The same type of response has been observed in the house fly (*Musca domestica*), the cray-fish (*Cambarus affinis*), and the lobster (*Homarus americanus*). The responses are sometimes of considerable magnitude; the eye of the house fly has given responses as great as 20 millivolts and more; whereas the largest responses of the frog eye rarely exceed 2.0 millivolts.

The response just described is typical in that it is the type most frequently obtained. There are, however, variations due to uncontrollable electric factors which cause fluctuations in the base-line of potential, independent of illumination. But the greatest variation in the electric response is brought about by relative differences in the form and magnitude of the A and B waves. Figure 2, *c* to *m*, is a series of different types of responses in which these relative differences are shown. In *c* the "A" wave recedes

Fig. 2. Types of electric responses to illumination obtained from arthropod eyes. *a–g*, responses to continued illumination (one second or more). *h–m*, responses to a flash of light, 0.060 second duration. *a*, response from excised lateral eye of a young horse-shoe crab, *Limulus polyphemus* (5 cm. across carapace). Illumination on eye, *ca.* 85,000 meter candles. Area of image 0.1 mm.^{2} Calibration at left, 1 step = 1.0 millivolt. *b–m*, responses from the compound eyes of various insects, obtained from the intact animals. *b*, from grasshopper, *Melanoplus femur-rubrum* 7. Calibration at left, 7.70 millivolts. Area of image, 0.17 mm.^{2} Illumination on eye 8,500 meter candles. *c*, from butterfly, *Vanessa atalanta* 1. Illumination on eye 8,500 meter candles. Area of image, 1.0 mm.^{2} *d*, from *Melanoplus femur-rubrum nymph* 10. Illumination of eye 85,000 meter candles. Area of image, 0.17 mm.^{2} *e*, from grasshopper, *Chortophaga viridisfasciata* 2. Illumination on eye 85,000 meter candles. Area of image 0.19 mm.^{2} *f*, from grasshopper, *Melanoplus differentialis* 3. Illumination on eye 8,500 meter candles. Area of image 0.17 mm.^{2} *g*, from *Melanoplus femur-rubrum* 2. Illumination on eye 85,000 meter candles. Area of image, 0.17 mm.^{2} *h*, from *Melanoplus femur-rubrum* 2. Illumination on eye 8,500 meter candles. Area of image, 0.17 mm.^{2} *i*, from *Melanoplus femur-rubrum* 7. Illumination on eye 8,500 meter candles. Area of image, 0.17 mm.^{2} *j*, from *Melanoplus femur-rubrum nymph* 9. Illumination on eye 8,500 meter candles. Area of image 0.17 mm.^{2} *k*, from *Melanoplus femur-rubrum* 3. Illumination on eye 85,000 meter candles. Area of image 0.17 mm.^{2} *l*, from *Melanoplus femur-rubrum nymph* 10. Illumination on eye 85,000 meter candles. Area of image 0.17 mm^2. *m*, from *Melanoplus differentialis* 3. Illumination on eye 8,500 meter candles. Area of image 0.17 mm.^{2} *a* and *b* are photographic records of the strong deflection; *c–m* are line tracings made from similar photographic records. In these tracings the calibration deflection has not been included—it would differ slightly in each case—the maxima of the separate curves represent potentials of 3 to 5 millivolts. Connections are so made that a current flowing in the external circuit from the "indifferent" electrode, on the animals' body (fig. 1, *b*, E_i), to the cornea of the eye (fig. 1, *b*, E_d) causes a deflection of the string shadow upwards in the records. The time record given for *c* is the same for all the others. The numbers are identification marks.

rapidly, often falling below the base-line; the "B" wave rises slowly, it may or may not be as large as the "A" wave, and it may or may not retain its maximum value throughout the duration of illumination. On the other hand, the recession of the "A" wave may be less marked and the rise of the "B" wave more rapid than in the "typical" response. Where the "B" wave attains a greater maximum than the "A" wave, we get the types figured in *d*, where the "A" wave is visible only as a slight notch in the rise of the "B" wave, and in *f*, where no "A" wave can be distinguished at all. This last type is important in the quantitative study, since it may easily lead to confusion. In still another case, the "B" wave may be very small, and unsustained; it then appears only as a "hump" on the recession of the "A" wave—as in *g*.

These differences are again brought out in the responses to flashes of light of very brief duration. Preparations showing a small "B" element in the response to continued illumination (as in fig. 2, *g*) react to a brief flash (less than 0.08 second) by a simple, spike-like wave of potential, rising to a maximum in the same time as does the "A" wave, and falling almost as sharply to the original base-line (fig. 2, *h*). When a well-marked "B" wave is present, as in the case of the typical response, *b*, the descending limb of the response to a flash is not so steep as in *h*, but the string returns to the base-line along a die-away curve, as shown in *i*. This is an effect presumably due to the presence of the "B" element of the response; it may even show itself as a slight hump in the descending limb of the curve, as in *j*, or may delay the return of the string to the base-line quite markedly, as in *k*. When the "B" wave of the response to continued illumination predominates, as in *d* and *f*, the response to a flash shows in turn either an element which tops the first wave of potential, as in *l*, or a simple rise of potential in which the "A" element is presumably completely masked by the "B" element as in *m*. In general the behavior of the "B" element runs parallel in both the responses to continued illumination and to a flash; thus *g* and *h* are obtained from the same individual, as were *b* and *i*, *d* and *l*, and also *f* and *m*. Evidence of the "B" element appears only in the responses to relatively long exposures (0.04 to 0.08 second); with extremely short exposures the responses are simple waves of potential, similar to *h* or *i*—presumably an uncomplicated "A" effect.

Thus these various types of action potentials are seen to be explicable on the basis of a relative difference, in time and magnitude, between two separate elements of potential change. What the factors governing these differences may be cannot be stated; the different types of response were all obtained from closely related organisms, from eyes which histologically present no marked differences: indeed, some of the most striking differences occurred between individuals of the same species. Thus the response figured in figure 2, *e*, is an exceptional case which can only with difficulty

be reconciled to the explanation given above. Yet this response, which shows what may be either an absence or a reversal of the "B" effect, is obtained almost without exception from the grasshopper, *Chortophaga viridisfasciata;* however, one single individual of this species gave a response similar to figure 2, *d*, and gave it consistently throughout a period of 48 hours. The grasshopper, *Melanoplus femur-rubrum*, moreover, has been observed to give responses of the types *b*, *d*, *f* and *g*, and one individual gave a response identical with *e*. A closely related grasshopper, *Melanoplus differentialis*, on the other hand, nearly always gives a response showing a predominance of the "B" wave—either type *d* or *f*. Even a given individual is apt to show changes in the type of response during an experiment lasting many hours. Thus a *M. differentialis* may change, in type of electric response, from *d* to *f;* or a *M. femur-rubrum* from *b* to *d* or to *g*. Fortunately, however, these changes are not great as a rule, and they are limited for the most part to the "B" element of the response.

QUANTITATIVE STUDY. In spite of the variations discussed the electric response of the arthropod retina may be studied quantitatively since the onset of illumination is followed by a fairly constant wave of potential (the "A" wave). It is the purpose of this section to present a study of the behaviour of the "A" wave, under constant conditions of temperature and in complete dark adaptation, as the stimulating light is varied.

In making such a study it is necessary to choose preparation where the "A" wave is not masked by the "B" wave, as in figure 2, *f;* it is moreover desirable to select experiments where the "B" wave does not even contribute, to any considerable extent, to the magnitude of the "A" wave. The eye of the grasshopper, *Melanoplus femur-rubrum* satisfies these requirements. The grasshopper's eye becomes completely light adapted in about 10 minutes, and requires about two hours to return to a state of dark adaptation (at about 20°C.). In the present investigation the state of adaptation of the eye was carefully controlled and the preparations were allowed at least three hours in the dark before any measurements were taken; after exposure of 0.2 to 1 second to a bright light at least thirty minutes were allowed to elapse before the experiments were carried further. A brief flash of light (less than 0.1 second) does not appreciably disturb the condition of dark adaptation, so that an interval of 5 or 10 minutes following exposure is an ample margin of safety.

The relation between the magnitude of the maximum values of the "A" and "B" waves and the intensity of the light whose onset produced them is shown in figure 3. The common logarithm of the intensity of the stimulating light (in arbitrary units) is plotted against the response in millivolts. The circles are values for the maximum of the "A" wave, the crosses are values for the maximum of the "B" wave. The "B" wave in this experiment maintained its maximum with great precision. At the lower inten-

sities the "A" wave becomes slow and feeble, and cannot be distinguished from the rise of the "B" wave. The preparation in this case was a bumble-bee. The curves are readily reproducible in other arthropods.

A logarithmic plot is used purely for convenience, since the range of intensity is very great, and since a direct plot would not bring out to a noticeable extent the "tailing off" which occurs at the low intensities. The closely rectilinear relation which the upper part of this curve shows when the data are plotted in this manner is convenient graphically, and interest-

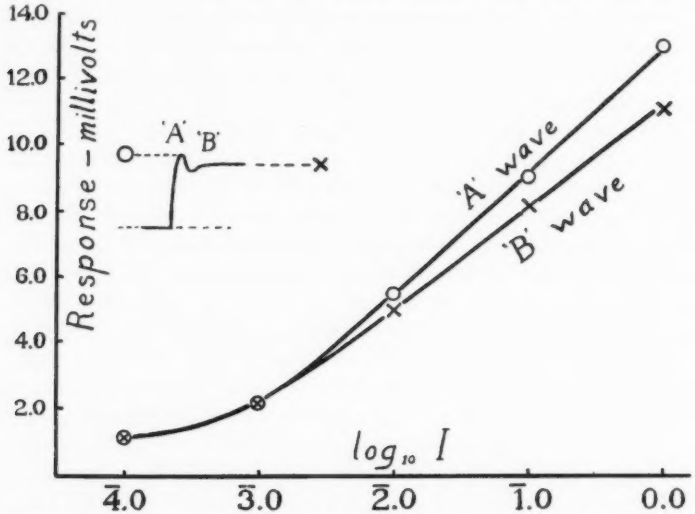

Fig. 3. Graph showing the relation between intensity of stimulating light and the magnitude of electric response to the onset of illumination. Ordinates, potential in millivolts; abscissae, common logarithm of the intensity of the illumination upon the eye, in arbitrary units such that I = 1 (log I = 0.0) corresponds to an illumination of 85,000 meter candles. Upper curve (circles), "A" wave, lower curve (crosses), "B" wave, as shown in insert. Area of image upon eye, 1.0 mm.^{2} From the compound eye of a bumble-bee (*Bombus pennsylvanicus*).

ing because it represents another instance of the so-called Weber-Fechner relation, that states that the effect in sensation is proportional to the logarithm of the intensity of the stimulus. This so-called law is in disrepute because it has been found that many other sets of data, set forth to support it, show, in reality, the very thing which this curve shows, *viz.*, that the relation does not hold except in a narrow range, and hence is only an approximation. This same type of relation holds in the case of the action potential of the frog eye, as shown by Chaffee, Bovie and Hampson (1, 1923), and by the present writer in an earlier paper (3). In the verte-

brate eye, however, the magnitude of response reaches a maximum value at the higher intensities, so that the curve tails off at the upper limit as well as at the lower, giving to the logarithmic plot the shape of an elongated S. It is quite probable that the same relation would be observed in the arthropod eye, were higher intensities available—the response could hardly

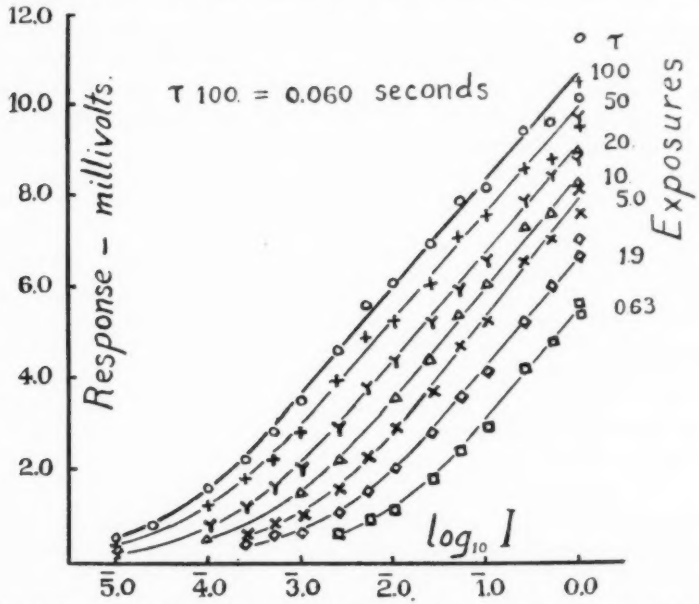

Fig. 4. Graphs showing the relation between intensity of stimulating light and the magnitude of electric response to a flash of light, for flashes of various duration. Each curve was obtained by plotting the potential developed by the eye in response to a flash of given, constant duration against the common logarithm of the intensity of the light flashed upon the eye. The duration of the flash is marked opposite the upper end of its respective curve; arbitrary units of time have been used, such that T 100 = 0.060 second. Ordinates, potential developed, in millivolts. Abscissae, common logarithm of intensity, in arbitrary units such that I = 1 (log I = 0.0) corresponds to an illumination of 85,000 meter candles. Area of image on eye, 0.17 mm.^{2} Temperature 25.0°C. Preparation, compound eye of *Melanoplus femur-rubrum* 5. Intact animal. The responses were similar to figure 2, *i*.

be expected to increase indefinitely with increasing intensity of stimulating light.

When the arthropod eye is illuminated by a very brief flash of light, not longer than 0.08 second to 0.10 second, the "A" effect is most marked, and in many preparations, especially in *M. femur-rubrum*, predominates entirely. The response in this case is then a simple wave of potential (fig. 2,

h or *i*), the magnitude of which represents almost entirely the magnitude of the "A" effect, and which varies with varying conditions of stimulation.

The curve marked τ *100* of figure 4 is the graph of the magnitude of the response obtained from the eye of an individual *Melanoplus femur-rubrum* upon exposing it for a constant length of time (0.06 second) to illumination of various intensities. The magnitude of the response, in millivolts, is plotted against the common logarithm of the intensity (in arbitrary units). The points are the experimental values of the magnitudes of the response to each intensity; the curve was drawn by eye to be the best fit for all the points. The data are from a single individual, and were obtained over a period of 18 hours, during which time the values of any given point could be reproduced within less than ± 5 per cent.

TABLE 1

Relation between intensity, I, of stimulating light, and exposure τ, necessary to produce a given constant response, E. The exposure, τ, is given in arbitrary units of time, where τ 100 = 0.060 second. The logarithm of the intensity of the light necessary to produce a given constant response is interpolated from the curves of figure 4, for each exposure. The intensity (in arbitrary units) is obtained from this logarithm and when multiplied by the exposure gives the value K^E, as described in the text. This procedure has been carried out for four different values of response.

E = 6.0 mv.			E = 4.0 mv.			E = 2.0 mv.			E = 1.0 mv.		
τ	$\log_{10} I$	K_E $(= I \cdot \tau)$	τ	$\log_{10} I$	K_E $(= I \cdot \tau)$	τ	$\log_{10} I$	K_E $(= I \cdot \tau)$	τ	$\log_{10} I$	K_E $(= I \cdot \tau)$
0.63	+0.19	0.98	0.63	−0.63	0.148	0.63	−1.50	0.0199	0.63	−2.16	0.0044
1.9	−0.28	1.00	1.9	−1.12	0.144	1.9	−1.97	0.0204	1.9	−2.64	0.0044
5.0	−0.76	0.87	5.0	−1.56	0.138	5.0	−2.39	0.0204	5.0	−3.05	0.0045
10.0	−1.00	1.00	10.0	−1.80	0.158	10.0	−2.68	0.0209	10.0	−3.38	0.0042
20.0	−1.32	0.96	20.0	−2.15	0.142	20.0	−2.99	0.0205	20.0	−3.76	0.0035
50.0	−1.69	1.02	50.0	−2.56	0.138	50.0	−3.43	0.0186	50.0	−4.12	0.0038
100.0	−2.00	1.00	100.0	−2.80	0.158	100.0	−3.72	0.0191	100.0	−4.46	0.0044

It will be seen that the same general type of relation exists in this case as was observed in figure 3, where the exposure was indefinitely long. When still shorter exposures are used, the same type of relation holds; figure 4 is, indeed, a family of curves each one of which represents the relation between the action potential and the intensity of light for a given constant exposure. The duration of the exposure is different for each curve, and is indicated in arbitrary units by the numbers printed above each curve. The units are such that τ *100* = 0.060 second.

Examination of this curve reveals the following points: 1, for any given constant value of the intensity of the stimulating light, shorter exposures yield smaller electric responses; 2, to produce any given, constant response a short exposure requires a brighter light than a long exposure. It is in the

quantitative expression of this second point that a most important and fundamental relation is brought out. Choosing a specific case, to produce a wave of action potential whose magniude is 6.0 millivolts, it will be seen from figure 4 that an exposure of 0.060 second (curve τ *100*), required an intensity of light whose logarithm to the base 10 is $\bar{2}.0$, which is 0.01 unit of illumination (arbitrary), while an exposure of 0.006 second (curve τ *10*) required a much brighter light, *viz.*, one whose logarithm is $\bar{1}.0$, which is 0.10 unit of illumination (on the same arbitrary scale). In this specific case a light of 0.01 unit of intensity shining on the eye for a period of 0.06 second produced a response of 6.0 millivolts, while to produce this same response by means of an exposure one-tenth as long, the light had to be made ten times as bright. To restate this finding more concisely one may say that to produce a certain constant response (6.0 mv.) the product of the intensity of the stimulating light into the length of time during which it shines must be constant:

$$0.010 \text{ unit of illumination} \times 0.060 \text{ second} =$$
$$0.10 \text{ unit of illumination} \times 0.006 \text{ second} = 0.0006.$$

Since the units representing intensity of illumination upon the eye can be reduced to meter-candles by multipying by a suitable factor, the figure on the right, 0.0006, can be reduced to meter-candle seconds, and represents, of course, the total energy per unit area received upon the eye during the flash.

Table 1, taken from figure 4, gives the results of similar calculations made for all seven exposures, choosing four values of response. The first column of each of the four sets is the value, in arbitrary units of time, of the duration of exposure; the second column gives the values of the logarithm of the intensity of the light necessary to produce the given response at each exposure, interpolated graphically from the smoothed curves of figure 4. From these two columns can be obtained the product of exposure into intensity, which is entered in column three. The smooth curves were drawn before any calculation was made; the interpolation of intensity was performed as in the above example.

From this table it will be seen that the product of intensity into duration of a flash of light necessary to produce a given response is, within reasonable limits of variability, constant. The table shows that this relation holds for four magnitudes of response, and for all the values of exposure and intensity used in this experiment. Mathematically this relation may be stated in the form

$$I \times \tau = K_E$$

where I is the intensity of illumination upon the eye, T the length of time during which it must shine to produce a given constant response of magni-

tude E; K_E is a constant (column 3, table 1), representing the amount of energy required to produce this response.

This experimental finding has been produced many times, both in different individuals of this species, and in individuals of different species. It is important to note, however, that this relation does not imply that the magnitude of the response is *proportional* to the product of intensity into exposure; it states that to produce a *given constant* response, this product must be *constant*. As a matter of fact, the relation between the energy of the flash ($I \cdot \tau$) and the magnitude of the response is quite different from a direct proportionality; to produce a response of 2.0 millivolt requires, by table 1, 0.020 unit of energy (on an arbitrary scale), while to produce a response of 4.0 millivolts requires not twice as much energy, but nearly eight times as much—*viz.*, 0.15 unit on the same scale. The relation between the energy of the flash and the magnitude of the response it produces is, as a matter of fact, given by any one of the curves of figure 4, for since the exposure is kept constant the energy of the flash is directly proportional to the intensity of the light. The energy of the flash at any given point on any one of these curves can be expressed in meter-candle-seconds by multiplying the intensity at that point by a number composed of two factors; the first reducing the arbitrary units of intensity of illumination per unit area of image to meter-candles per unit area, the second expressing the exposure, which the particular curve represents, in seconds. This number is constant for all the points on any given curve, and since the intensity is plotted as the logarithm, multiplying by this number is done by adding the logarithm of the number to the values of all of the abscissae, hence the curve is merely shifted along the X-axis to the right or left. The exposure is different for each curve, but since the relation brought out above is known to hold, it follows that each curve will be shifted just enough to superimpose all of the curves upon one another, so that all of the points will lie on a single curve. This single curve is then the graphic representation of the function (E, log H), where E is the response in millivolts, and H the energy in meter-candle-seconds per unit area. Since the area of the image on the eye is constant in this experiment (0.17 sq. mm.), the total energy received by the eye is proportional to H.

This curve relating energy of flash to response brings us to the next consideration. By the same reasoning as was carried out above, it is obvious that the energy of a flash of light of any given constant intensity must be proportional to the duration of the flash. Hence a curve relating magnitude of response to the logarithm of the duration of exposure at constant intensity is identical with the curves relating the magnitude of response to the logarithm of the intensity of the light at constant exposure, except that it is shifted by the proper amount to the right or left. Thus a family of curves, directly comparable to figure 4, but representing the effect of ex-

posure upon response, at various given intensities, can be obtained from each set of data. But it is known, from figure 3, that the "A" wave in indefinitely long exposures of light is a function only of the intensity of the light. A light of given intensity can produce an "A" wave of only a certain value, no matter how long it may shine: to increase the magnitude of the "A" wave the *intensity* of the stimulus must be increased. Thus each curve relating response to exposure, in the family mentioned above, must have an upper limit, beyond which an increase in exposure will cause no further increase in magnitude of response. The value of this maximum response will depend upon the intensity of the light for which that particular curve was drawn. It therefore follows that the reciprocal relation between intensity and exposure must have certain limitations. That there must be some sort of limitation is of course apparent from the nature of the response in question. It will be remembered that the "A" wave of the "on effect" reaches its maximum value in 0.10 second to 0.12 second after the onset of illumination, and then subsides, even though the light continues to shine. Hence light shining for any period greater than 0.10 second to 0.12 second cannot produce an "A" wave any greater than that produced by a flash of light which lasts only until this maximum value is attained. As a matter of fact, experiment shows that the period during which light is effective in contributing to the magnitude of the "A" wave is somewhat less than the time required for the potential to reach its maximum value. It has been found that the exposure which will just barely produce a maximum "A" wave at a given intensity has the same energy content as that required to produce an equal response by a flash of greater brightness shining for a shorter time. The value of this exposure, beyond which the light is no longer effective in increasing the magnitude of the response, and above which the reciprocal relation between intensity and exposure can no longer hold, is about 0.06 second to 0.08 second. The experimental data accumulated thus far indicate that this "critical" exposure is relatively constant for all intensities, but as they are none too clear-cut on this point the whole question of the limitation of the $I \cdot \tau$ relationship may be discussed only after further experimentation.

Discussion. The quantitative study of the first part of the response of the arthropod eye to illumination, in spite of the complexity one might expect to encounter, has yielded one simple, clear-cut relation. The expression of this relation states that in order to produce an electric response of given, constant magnitude, to a brief flash of light, the energy of that flash, as given by the product of its intensity into its duration, must be constant. This relationship is well known in visual physiology, and has its counterpart, and perhaps its basis, in photochemistry as the Bunsen-Roscoe law.

The Bunsen-Roscoe law states that in a simple, irreversible photochemi-

cal system the amount of energy, as given by the product of the intensity of the light into the duration of exposure, must be constant in order to produce a given constant amount of photochemical change. This law has had some application in biology, and may be applied to advantage in this case, if one assumption be permitted. This assumption is that the first step in the process which results in an action potential is the action of the light upon a photochemical system in the retina, and that a given amount of photochemical change in this system will always result in an electric response of constant corresponding magnitude. The reciprocal relation which has been found to exist between intensity and exposure may then be ascribed to a known physical law governing all photochemical reactions in general and in particular the photochemical reaction in the sense-organ which results ultimately, through an unknown chain of processes, in the "A" wave of the "on effect."

The fact that there is a "critical" exposure, beyond which light of a given intensity is no longer able to increase the magnitude of the response, places a limit to the applicability of the Bunsen-Roscoe law in this particular case, but by no means detracts from its possible significance. It is explicable on the assumption that the chain of processes which result ultimately in the "A" wave are set going at the end of a "sensitization period," equal in length to the "critical exposure;" that the magnitude of the resulting electric response is determined by the amount of photochemical change which has taken place by the end of this sensitization period and which is therefore proportional to the amount of energy received during the sensitization period only. The response would be unaffected by continued illumination, once these processes were set going, and figure 3 would represent one of the family of curves of figure 4, where the exposure, instead of being limited by a shutter is limited by the sense-organ, and is τ_s, the length of the sensitization period. This last statement would hold only if τ_s be constant at all intensities.

The nature of the entire process, of which the photochemical reaction is the first step and the electrical response the last, is of course unknown. Preliminary experiments indicate, as would be expected, that it is by no means simple.

The assumption, based on just such a Bunsen-Roscoe relationship, that a photochemical reaction is the first step in photoreception is by no means new to visual physiology. It is important, however, that this assumption, with proper limitations, be brought forward by such experiments as these, for it will serve to make a beginning in the analysis of the present problem.

SUMMARY

1. The electric response of the eye to light stimulation has been studied in various arthropods.

2. The electric response to the onset of illumination consists of a change in potential in such a direction that the cornea of the eye becomes more negative, with respect to the back of the eye, than it was prior to illumination.

3. In *Limulus polyphemus* the response is a simple wave of potential, which attains a maximum in less than 1 second, and then falls off asymptotically even during continued illumination.

4. In most other arthropods (chiefly insects) the potential change in response to the onset of illumination consists of two waves: an "A" wave, having a maximum in 0.10 to 0.12 second after the onset of illumination, followed by a "B" wave which reaches and generally maintains a maximum in less than 1 second.

5. Variations from the typical response may be explained in terms of relative difference in form and magnitude of these two waves.

6. The response to cessation of illumination is a simple decay of potential, asymptotic to its value before the onset of illumination.

7. A quantitative study of the "A" wave has been made in certain insects under varying conditions of stimulation and constant conditions of temperature and adaptation.

8. It has been found that in order to produce a wave of potential of given constant magnitude the energy of the stimulus, as given by the product of intensity of the light into duration of exposure, must be constant.

9. This relation holds only for exposures less than 0.06 second to 0.08 second. Exposures longer than this yield a maximum "A" wave, the magnitude of which depends on the intensity alone.

10. These facts may be explained by assuming that the first step in the process which results in an action potential is the action of light upon a photochemical system in the photoreceptor, and that a given amount of photochemical change in this system results in an electric response of given corresponding magnitude. The observed relation between intensity and exposure is then the consequence of the Bunsen-Roscoe law, applying to the photochemical system.

I wish to express my appreciation to Prof. C. D. Snyder, under whose friendly supervision this work was carried out, for advice and help at many points, and for furnishing opportunity and equipment.

BIBLIOGRAPHY

(1) CHAFFEE ET AL. 1923. Journ. Opt. Soc. Amer., vii, 1.
(2) CHAFFEE ET AL. 1924. Ibid., ix, 1.
(3) HARTLINE, H. K. 1925. This Journal, lxiii, 600, 612.

THE EFFECT OF ELIMINATING THE SPHINCTER OF THE COMMON BILE DUCT UPON EMPTYING OF THE GALL BLADDER

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Received for publication October 22, 1927

In spite of recent evidence obtained by different methods that the gall bladder expels its contents by muscular contraction during digestion (1), (2), (3), (4), (5), (6), (7), (8), (9) some investigators still hold that the active factor in emptying the gall bladder is the sphincter of the common bile duct, the rôle of the gall bladder being passive; emptying by its normal tonus and elasticity when the sphincter releases (10), (11), (12), (13), (14), (15), (16).[3]

The obvious way to settle the question as to whether or not the sphincter of the common bile duct plays a definite rôle in emptying the gall bladder is to eliminate it and note the effect. It has been reported by one of us (6), (7) that after dilating and cutting the common-duct sphincter in cats the gall bladder will remain full for days, and then empty normally after feeding. Berg and Jobling (17) also have shown that transplantation of the common duct had no effect upon the emptying of the gall bladder in the dog. This seems to disprove the theory that the gall bladder empties by its elasticity with release of the common-duct sphincter. However, in the case where the sphincter is cut or the duct transplanted there may be some question as to whether the musculature of the duodenum does not still maintain a slight sphincteric action.

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[3] Anatomically there may be a separate sphincter at the end of the common bile duct, although a true sphincter is denied by Burget, also Copher and Kodama. Recent evidence that there is a sphincter, independent of the duodenal musculature, has been obtained by Mann and Higgins (Proc. Soc. Exper. Biol. and Med., 1927, xxiv, 533). However this may be, there is no doubt of the presence of a sphincteric action, and the point of entrance of the common duct into the duodenum is referred to in this paper as the common-duct sphincter. When a dilating probe is run into the sphincter from the duodenal side a high resistance is encountered. When dilated, however, the tonus of the sphincter does not quickly return. One receives the impression that a "catch mechanism" such as Bayliss supposes for the contraction of the adductor muscle in bivalves, is active here.

More direct evidence has been obtained by Boyden and Birch (18) that the gall bladder empties independently of any action of the duodenum. Using a method devised by one of us, they filled the gall bladder of a cat with iodized oil for observation by x-ray (1), (2), (6), and then cut the common bile duct. There was slight spontaneous emptying but the next day the gall bladder had refilled. It then partially emptied in response to food, obviously without being influenced by any action of the duodenum.

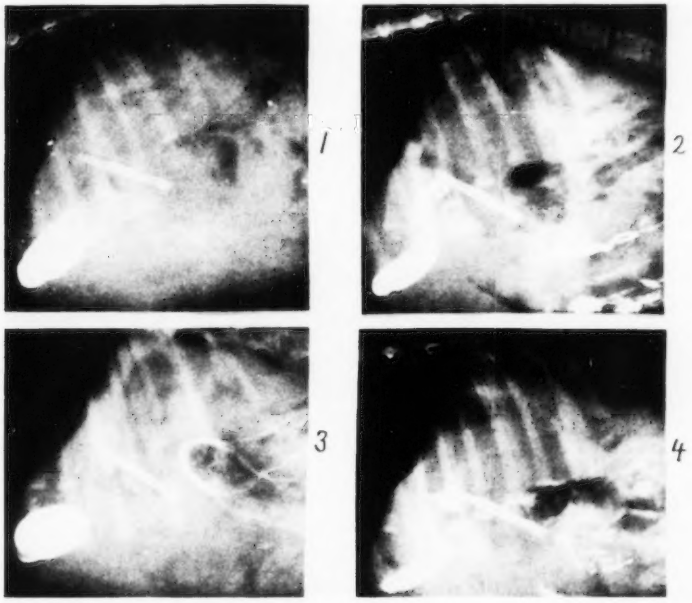

Fig. 1. Roentgenogram of a puppy five hours after the gall bladder had been filled with iodized oil and a glass cannula inserted, from within the duodenum, through its wall, into the common bile duct, eliminating any sphincteric action of the musculature about the termination of the duct. Shadow of the cannula is seen just above the shadow of the gall bladder. Previous roentgenograms showed neither changes in shape nor expulsion of oil over a period of three hours. The notch in the fundus indicates the point of closure of the opening for injection of the oil.

Fig. 2. Four and a half hours after feeding egg yolks, there is an elongation of the gall bladder (due probably to the action of its circular muscle fibres) with considerable emptying. Note the iodized oil in the cannula and in the intestine.

Fig. 3. Same animal as in figure 1, twenty-two and a half hours after the first feeding. The gall bladder has completely relaxed and refilled with bile, enough iodized oil remaining to make it visible. The puppy was then fed two egg yolks by tube.

Fig. 4. Five and a half hours after the second feeding approximately ninety per cent of the contents of the gall bladder has been expelled independently of any sphincteric action at the termination of the common bile duct.

To obtain further evidence upon this question, we decided to perform an experiment with an inlying glass cannula at the entrance of the bile duct into the duodenum, extending through the intestinal wall, thus eliminating any effect of tonus in the duodenum or the common-duct sphincter. In a dog the duodenum was opened, the sphincter dilated and cut, the cannula inserted and held with a stitch. The duodenum was then sewed up, the gall bladder filled with iodized oil, the abdomen closed, and the animal allowed to recover.

In this way an experimental subject more nearly normal than that of Boyden and Birch was obtained, who allowed the liver bile to discharge into the peritoneal cavity.

It should be re-emphasized that an animal must be in excellent condition in order that the gall bladder empty normally. Any anesthetic, any exposure of the viscera or even illness of the animal may inhibit the motor activity of the gall bladder (6), (7).

The accompanying figures show that in the normal dog with the sphincter eliminated the gall bladder may expel at least ninety per cent of its contents in a few hours in response to the ingestion of fat. It is difficult to see how any other agent than the musculature of the gall bladder itself could effect this result.

It will be noted that in this case the gall bladder refills normally between feedings, which does not ordinarily occur with the common-duct sphincter out of action. In all probability the refilling of the gall bladder here, in spite of the apparent inactivity of the sphincter, is due to the bile meeting the slight obstruction of the cannula in the common duct, and backing up into the gall bladder when it is relaxed during fasting. When the gall bladder is active during digestion it apparently exerts sufficient pressure to push the bile and oil out through the cannula.

Other experiments similar to that described above were performed on the cat with like results.

CONCLUSIONS

With any sphincteric action of the intestinal musculature at the termination of the common bile duct prevented by an inlying cannula the gall bladder shows no emptying for hours; but when the animal is fed, it expels its contents in a normal manner. Consequently, it may be assumed that the release of the sphincter of the common bile duct does not result in expulsion of the contents of the gall bladder by its elasticity, but that some other factor, presumably muscular contraction, is responsible for its emptying.

BIBLIOGRAPHY

(1) WHITAKER, L. R. 1926. Boston Med. Surg. Journ., cxciv, 373.
(2) WHITAKER, L. R. AND E. A. BOYDEN. 1926. This Journal, lxxvi, 199.
(3) McMASTER, P. D. AND R. ELMAN. 1926. Journ. Exper. Med., xliv, 173.
(4) BOYDEN, E. A. 1926. Anat. Rec., xxxiii, 201.
(5) HIGGINS, G. M. AND F. C. MANN. 1926. This Journal, lxxviii, 339.
(6) WHITAKER, L. R. 1926. This Journal, lxxviii, 411.
(7) WHITAKER, L. R. 1927. Journ. Amer. Med. Assoc., lxxxviii, 1542.
(8) MANN, F. C. AND G. M. HIGGINS. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 931
(9) HAMRICK, R. A. 1927. Am. J. Med. Sc., clxxiv, 168.
(10) BURGET, G. E. 1925. This Journal, lxxiv, 583.
(11) COPHER, G. H. AND S. KODAMA. 1926. Journ. Exper. Med., xliv, 65.
(12) KODAMA, S. 1926. This Journal, lxxvii, 385.
(13) BURGET, G. E. 1926. This Journal, lxxix, 130.
(14) GRAHAM, E. A. 1926. Amer. Journ. Med. Sci., clxxii, 625.
(15) GRAHAM, E. A. 1927. Surg., Gyn. and Obs., xliv, 153.
(16) BURGET, G. E. 1927. This Journal, lxxxi, 422.
(17) BERG, B. N. AND J. W. JOBLING. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 434.
(18) BOYDEN, E. A. AND C. L. BIRCH. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 827.

THE RESPONSE OF THE ISOLATED SEGMENT OF SMALL INTESTINE (RABBIT) TO EXTRACTS OF YEAST AND OTHER SUBSTANCES DURING THEIR PASSAGE THROUGH THE LUMEN

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Received for publication October 5, 1927

In this investigation, a continuous perfusion method was used. Comparison of effects was made with the Magnus (1) strip and with this method used essentially as that of Trendelenburg (2).

The apparatus employed provides for the maintenance of a constant pressure within and a continuous flow of a liquid through a vertically suspended segment of intestine while records of the longitudinal contractions and volume changes are being simultaneously recorded on a smoked drum. It further provides two reservoirs one containing fluid for a control record, the other a solution of the drug or extract under consideration, for an experimental record. The fluids are readily replaceable one by the other without interrupting the continuity of the record or altering the behavior of the segment in a mechanical sense.

Apparatus. For the convenience of description, the apparatus consists of an inlet and an outlet system, the division occurring at the segment. Figure 1 is a schematic drawing of the essential parts. Fluid leaves the Wolff bottle, *W.B.*, and passes through the valve, *F.V.*, into a coil immersed in the constant temperature bath. It then passes through one or the other of the arms of the stationary glass piece, *S.G.P.*, into the common arm, *C.A.*, which extends into the beaker of salt solution and thence through the segment, *S*. In the outlet system the fluid escapes at a slow rate of flow at the exit, but due to a constriction at *C.C.* it rises in the graduated side-arm, *G.S.A.*, which indicates the pressure to which the segment is subjected.

The stationary glass piece affords by virtue of the three-way stopcock a ready means of replacing one fluid by the other. The common arm reaches to the bottom of the beaker and then turns up and very shortly ends in a lip by means of which the oral end of the segment is held in place. The two inlet arms at the upper side of the stopcock fit into a clamp which holds the whole piece firmly. Each arm receives fluid from a separate reservoir, *W.B.*

The valve, *F.V.*, is arranged to prevent the backward flow of solution when the segment is actively contracting. It is constructed of a strip of rubber membrane covering the opening of a piece of glass tubing, and is arranged in a small glass chamber. When the circular fibers are vigorously contracting, the fluid has two paths of escape either back into the inlet system or into the outlet system where it rises in the side arm. This valve checks the backward flow thus allowing the full effect to be exerted on the side arm. The tambour in communication with the side arm records the volume change minus an insignificant volume which passes through the exit tube.

The Wolff bottle, *W.B.*, contains the perfusion fluid and maintains constant pressure within the segment. It is arranged after the manner of the

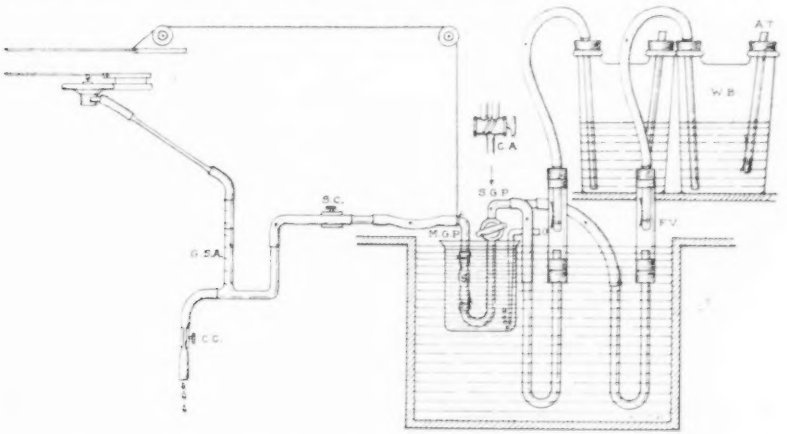

Fig. 1. Schematic drawing of the essential parts of the apparatus used.

Marriotte bottle for constant pressure. Either may be used. When one bottle is not operating, the fluid tends to rise in the tube, *A.T.*, leading to the atmosphere. If this were allowed to remain until the system replaced the other one, a difference in pressure would be recorded. This difference gradually becomes smaller as the fluid drops in the tube to the level of the opening. To eliminate this a valve is placed over the opening at the lower end of the tube permitting the inflow of air into the bottle but preventing the rise of fluid in it. This arrangement affords an even record in the tracings of the longitudinal and circular contractions when one fluid replaces the other.

In the outlet system the movable glass outlet tube, *M.G.P.*, has a lip on its lower end by means of which the aboral end of a segment is held in place. The upper end is bent at a right angle and is connected by means

of a flexible rubber tubing to a piece of glass tubing which is held in place by means of the stationary clamp, *S.C.* The rubber tubing permits the full effects of the longitudinal contractions to be recorded. At the bent end, a piece of thread is attached and brought over a system of pulleys to a lever which records the contractions of the longitudinal fibers.

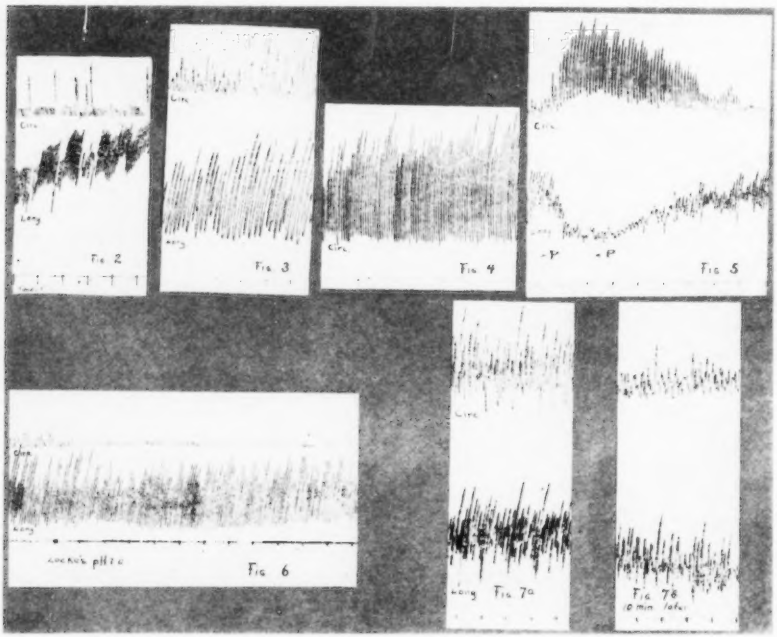

Fig. 2. Rising tonus in the longitudinal fibers of a duodenal segment without change in the diastolic level. Time is in minutes in all figures.

Fig. 3. Increase in amplitude of the longitudinal fibers does not alter the diastolic level.

Fig. 4. Gradual increase in volume output attended with constant diastolic level.

Fig. 5. Effect of pressure differences in a segment of jejunum.

Fig. 6. Effect of Locke's solution at pH of 7.0 on a segment of jejunum.

Fig. 7. Effect of Locke's solution pH 6.0 on ileal segment. a-control record. b-record 10 minutes after introducing acid solution.

The glass piece with graduated side arm is represented at *G.S.A.* The side arm is calibrated to give the volume change. The tube connecting the side arm and tambour has a fine bore to diminish the air space. The constriction on the exit tube can be made either larger or smaller by the constricting clamp, *C.C.*, depending upon the rate of flow desired.

Pressure considerations. Since this method depends upon the maintenance of an even pressure, the factors involved need some explanation. The resting level of the fluid in the side arm and consequently of the tambour record on the drum is designated as the diastolic level of either volume changes or of circular contractions. To this level both bottles are carefully adjusted. There are a number of conditions which at first sight may seem to modify this level, but the accompanying figures show how remarkably constant it is held. Figure 2 illustrates a rising tonus in the longitudinal fibers, yet the diastolic level is not altered. The rise in tonus is a normal occurrence and is found very often in duodenal or jejunal segments of the rabbit immediately after suspension. The same holds true when the tonus of the longitudinal fibers is falling. This does not mean that each contraction of the circular fibers is followed by the same degree of relaxation, for tonus variations exist. The general diastolic level, however, remains the same. If the amplitude of the longitudinal contractions progressively increases without a material increase in tonus, as ileal segments often show, the volume changes are usually increased but again the diastolic level remains the same. Figure 3, taken from the ileum of a rabbit, illustrates this point. The converse of this is true. If the amplitude of contractions is steadily declining, the output of fluid by the segment usually falls, but the diastolic level remains the same. This increase and decrease in amplitude of contractions of the longitudinal fibers without affecting the general fluid level is also seen in the circular contractions. Figure 4 shows a gradual increase in volume output. The converse of this is likewise true.

The evidence outlined above demonstrates that tonic conditions or variations in volume output from the segment are without influence upon the diastolic level in the side arm. The most important factor is the control of the pressure. Maintenance of an even pressure throughout the entire experiment is absolutely necessary for maintenance of constant diastolic level. Figure 5, taken from the jejunum of the rabbit, illustrates the effect on both muscular coats of pressure differences. With an increase in pressure, the volume output is enormously increased while the longitudinal contractions decrease in amplitude and the segment elongates. When the pressure is allowed to fall slowly, the volume output gradually decreases while the longitudinal contractions increase in amplitude and the segment shortens. From this figure it is seen that at one pressure the contractions occur chiefly in the circular fibers while at another they are in the longitudinal fibers. The aim is to strike an intermediate pressure under which contractions of both muscular coats can be recorded.

Preparatory procedure. Before an actual experiment is begun, a short piece of rubber tubing is attached to the apparatus in the place of a segment for the purpose of equilibrating the pressures in both bottles. The

aim is to replace one fluid by the other with an imperceptible change in pressure. A disturbing factor is bubbles in the system. These must be removed from the entire apparatus. A knowledge of the pressure which is feasible to work with is gained by experience. The pressure of one bottle is adjusted to a certain height in the side arm. At this time the rate of perfusion is established and once it is determined it cannot be altered during the course of an experiment without altering the pressure. The fluid is allowed to flow for some minutes to insure a constant level in the side arm. The stopcock is then turned to permit fluid to pass from the other bottle. The height of the fluid column in the side arm is brought to the value of the first bottle by either raising or lowering the other. The fluid is allowed, as mentioned above, to flow for several minutes to insure a constant level in the side arm. The stopcock is then turned back and forth a few times to make it certain that no minor differences in pressure exist. Finally, the plain salt solution is allowed to wash out the common arm. The stopcock is closed and the exit tube pinched off to prevent the fluid falling in the side arm. The piece of rubber tubing between the inlet and the outlet systems is detached. The apparatus is now ready for an actual experiment.

Experimental procedure. A segment of intestine about 6 cm. long is thoroughly washed out with Locke's solution. The importance of thorough washing cannot be over emphasized, for the accumulation of suspended material anywhere in the system seriously interferes with the smoothness of operation. The segment is carefully tied at its oral end by a piece of clean thread to the stationary glass tube and at its aboral end to the glass outlet tube. This procedure permits the fluid to pass in its normal direction through the segment. Furthermore, peristaltic waves pass over the segment in the aboral direction and the fluid displaced readily rises in the side arm to register in the tambour. The segment with its attachments is immediately immersed into a beaker of oxygenated Locke's solution kept at 38°C. Locke's solution is allowed to enter the segment very gently until the full pressure is exerted upon it. The clamp on the exit tube is removed at the same time. After a variable period of time during which the segment is becoming adjusted to its new environment, a control record can be taken. The levers are arranged in order one beneath the other while the segment is assuming a good beating rhythm. It is necessary to have the pulleys in line so as to offer as little resistance as possible to the longitudinal contractions. The segment is carefully weighted to prevent lag of motion. A control record is taken for about 30 minutes, or longer if necessary, and when the tracings show that the segment has reached a state of equilibrium, the stopcock is quickly turned when the segment is fully relaxed, to the other system containing Locke's solution plus the drug or extract studied. The time required for

the fluid to pass from the stopcock to the segment will obviously depend upon the rate of flow. The experiment can be continued for several hours depending upon the condition of the segment and upon the substance studied. Thorough and even oxygenation of the Locke's solution in the beaker must be maintained throughout the experiment. A recovery period can be run on the segment by turning back to the plain Locke's solution.

The effect of various tensions of carbon dioxide in the perfusion fluid. There is a difference of opinion as to whether or not carbon dioxide stimulates the activity of the intestines. Bokai (3) was able to set up peristaltic activity of the intestine by injecting carbon dioxide gas into the lumen. Hooker (4) observed that carbon dioxide gas had a stimulating effect upon arrhythmic strips of intestine exposed in a moist chamber. Uniform depression, however, resulted where rhythmic activity was present. Young (5) employing the Magnus (1) method was never able to observe any stimulating effect of the gas upon the intestines of rabbits and cats. Fraser (6) performing some careful experiments in which the acidity of the solution bathing the segment was increased by carbon dioxide, demonstrated a cessation of all rhythmic movements at a pH of 6.4. The segments fully recovered in normal solution.

Since, in the method outlined above, the acid solution can be brought in contact with the intestinal mucosa, comparisons can be made with the results obtained by the Magnus method. Carbon dioxide gas from a Kipp generator was used to produce the various degrees of hydrogen ion concentration of the perfusion fluid. pH determinations were made by Clark's (7) colorimetric method.

Figure 6 illustrates an experiment on the jejunum of the rabbit with Locke's solution at a pH of 7.0. A normal record was obtained with Locke's solution at a pH of 7.4. There is a diminution in the volume output, but the tonus and rate and amplitude of contraction of the longitudinal fibers remain practically the same. No peristaltic waves appeared either in the control or the experimental period.

Figure 7, a, and b, illustrates the effect upon a segment of ileum of changing to Locke's solution at a pH of 6.0. The volume output is considerably decreased, and the rhythmical activity of the circular fibers is interfered with. No peristaltic waves appear at any time. The longitudinal fibers slowly lose in tonus, amplitude, rate and regularity of contraction. The pH of the Locke's solution in the beaker at the end of the experiment was 7.3.

In figure 8 Locke's solution, pH 5.3, was passed through the segment of ileum. The striking effect 25 minutes later is the great reduction in amplitude of the longitudinal fibers. At the same time, the volume output is considerably decreased and contractions of the circular fibers

are irregular. When Locke's solution at a pH of 7.4 was substituted for the acid solution the segment 42 minutes later showed a tendency to recover but never reached the original state.

The experimental evidence outlined above clearly indicates no beneficial action of carbon dioxide in solution under the various tensions on the muscular activity of the intestinal segment. If peristaltic waves were present in the control period, they were obliterated in the experimental period. Carbon dioxide did not arouse any waves when they were absent. In view of the fact that Fraser (6) by the immersion method obtained cessation of movements at a pH of 6.4, the figures referred to above are significant in that they demonstrate a greater impenetrability of the mucosa to carbon dioxide than the serosa. Segments having an acid solution as low as a pH of 5.3 did not entirely cease beating and also showed some tendency to recovery.

The effect of barium chloride. Barium chloride has been known to cause powerful contractions in smooth muscle tissues. Bauer (8) employing the Trendelenburg (2) method and concentrations of barium chloride compatible with physiological regularity of function of the longitudinal and circular fibers, demonstrated that the salt effect parallels the strengthening of peristalsis produced by an increase in the volume of the intestinal contents.

Since the method described permits the study of substances in contact either with the mucous or the serous membrane, it was thought to be important to compare the effects applied in the two ways of a drug whose action is so well known. The duodenum of a rabbit was used. Figure 9 is an experiment in which the concentrations of barium chloride necessary for a distinct effect was determined. When a satisfactory control record was obtained, barium chloride solution was introduced into the beaker to make a concentration of 1 to 100,000. The effect was immediate. The tonus of the longitudinal fibers was increased, the amplitude became much greater, but the rate fell from 14 to 13 per minute. The circular fibers

Fig. 8. Segment of ileum. a-Locke's solution pH 5.3 substituted. b-25 minutes later, Locke's solution pH 7.4 substituted for the acidified solution. c-42 minutes later, showing recovery.

Fig. 9. Effect of barium chloride in contact with serous membrane of duodenal segment. The large spikes in the upper record denote peristaltic waves.

Fig. 10. Barium chloride in Locke's solution passing through a segment of duodenum.

Fig. 11. Effect of autolyzed yeast on the duodenum (perfusion method).

Fig. 12. Effect of a water extract of baker's yeast on the jejunum (Magnus strip).

Fig. 13. Fifty-two per cent alcoholic extract of autolyzed yeast on a segment of ileum.

Fig. 14. Fraction two on a segment of ileum.

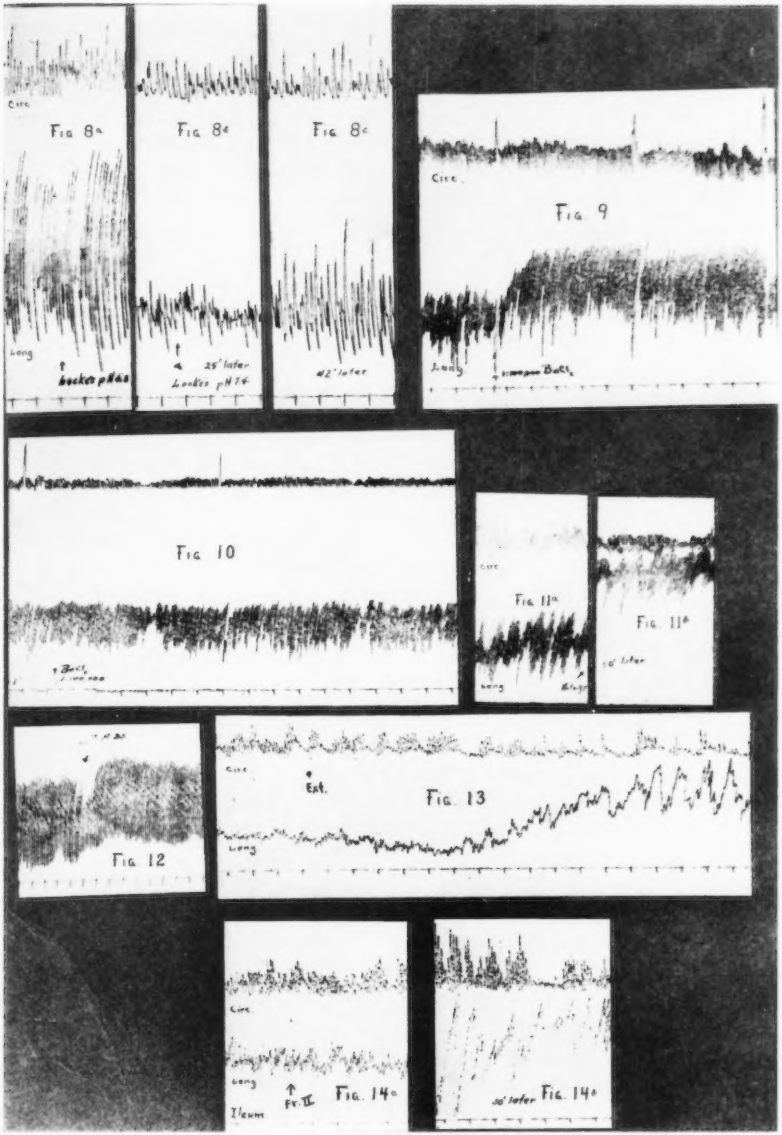
Fig. 8a
Fig. 8d
Fig. 8c
Fig. 9
Fig. 10
Fig. 11a
Fig. 11b
Fig. 12
Fig. 13
Ext.
Fig. 14a
Fig. 14b

Figs. 8–14

responded at first with a peristaltic wave and then with a greater output of fluid. The peristaltic waves, shown by the large spikes on the upper record, which were absent in the control period, continued at regular intervals. The gradual fall in the fluid level is due to a leak in the air system between the side arm and the tambour.

Knowing the concentration of barium chloride necessary to produce a distinct effect, the salt was used in the perfusion fluid but not in immersion on a segment contiguous to the above. Figure 10 illustrates this experiment. There is no effect on the tonus of the longitudinal fibers but a very slight increase in amplitude may be read from the tracing. The rate increased from 14 to 15 per minute. The circular fibers were not affected. The peristaltic waves, which occurred at regular intervals during the control period, became more infrequent and less vigorous.

From this experiment it is evident that barium chloride at a concentration of 1 to 100,000 produces a distinct effect when it is applied to the serous membrane. It confirms the findings of Bauer (8). On the other hand, when the salt passes through the lumen of the segment at the same concentration, no definite effects are noticed.

Yeast (Saccharomyces cerevisiae). In studies on human beings and on experimental animals, yeast has been shown to exert an influence upon the motor function of the intestines. The work on this subject has been summarized by Murlin and Mattill (9). Gross (10) while investigating vitamin deficiencies in rats found that marmite added to the diet of vitamin B deficient rats, not only caused the charcoal marker to appear in a much shorter time, but hastened its complete elimination. Due to the fact that the effect was produced after several days, he did not ascribe to yeast any local stimulating action. In isolated segments of intestine in isotonic and hypertonic solutions of yeast, he obtained inhibition.

In this investigation an attempt was made to determine the action of yeast on intestinal motility and to isolate the active principle. Baker's yeast (Fleischmann's) was used. Rabbits served as experimental animals. The perfusion method described above was employed and comparisons made with the results obtained by the Magnus (1) and the Trendelenburg (2) methods. Hydrogen ion concentration was determined by Clark's (7) colorimetric method and all solutions were adjusted to a pH of 7.4.

Figure 11, taken from the duodenum, represents a typical result of a water extract of autolyzed yeast. Similar results are obtained with water extracts of fresh yeast. The chief effect noted here is an increase in tonus without an increase in rate. The volume output is diminished, a fact often observed where there is an increase in tonus. No peristaltic waves appeared during the control period and none were initiated by the extract. With the Magnus method, the effect of a water extract on the jejunal segment in figure 12 is immediate. The tonus is permanently

raised with no change in rate. In ileal segments the tonus rise is not so marked, but the amplitude and rate are slightly increased as a rule.

Figure 13 illustrates a typical result with a 52 per cent alcoholic extract of autolyzed yeast on a segment of ileum. In a short time after the introduction of the extract, the tonus of the longitudinal fibers is raised, and large tonus waves induced. The rhythmical contractions of the circular fibers, however, become less frequent and less regular.

Yeast was further analyzed by the method of Osborne and Wakeman (11). Fraction 2 with which they obtained most favorable growth gave the greatest stimulation to intestinal activity. With the Magnus strip, as a rule, it increased the tonus. In the perfusion experiments, a typical picture is obtained from figure 14. The first result is a stimulation of the circular fibers followed by less vigorous movements as the tonus of the longitudinal fibers rises and as tonus waves appear. The other fractions are relatively inactive indicating that the stimulating agent resides in fraction 2. It is to be remembered that this fraction bears the greatest amount of vitamin B.

The chief effect of yeast, then, is upon tonus. With the Magnus method, the increase in tonus is greatest in duodenal and jejunal segments whereas in ileal segments the amplitude increases. There is no constant finding as regards the effect on rate of contraction. Ileal segments more often showed an increase while duodenal segments slowed up when the tonus rose considerably. Insofar as the effect is brought about by fraction 2, it is probable that vitamin B is the stimulating agent.

SUMMARY

A method for the study of the muscular activity of the intestinal segment permitting a continuous flow through its lumen of a fluid which is readily replaceable by another is presented. Longitudinal contractions and volume changes due to contractions of the circular fibers and to peristaltic waves are simultaneously recorded.

Evidence is presented indicating no beneficial effect of carbon dioxide at various tensions upon the muscular activity of the segment.

Barium chloride is shown to have a distinct stimulating effect in a concentration of 1–100,000 when applied to the serous membrane, but no effect when it was passed through the lumen.

Yeast (Saccharomyces cerevisiae) is shown to have a definite stimulating effect on tonus. Evidence is presented for the assumption that the effect is due to vitamin B.

The author wishes to express his gratitude to Dr. John R. Murlin for his helpful suggestions during the course of this investigation.

BIBLIOGRAPHY

(1) MAGNUS, R. Pflüger's Arch., 1904, cii, 123.
(2) TRENDELENBURG, P. Arch. f. exper. Path. u. Pharm., 1917, lxxxi, 55.
(3) BOKAI, A. Arch. f. exper. Path. u. Pharm., 1887, xxiv, 153.
(4) HOOKER, D. R. This Journal, 1912, xxxi, 47.
(5) YOUNG, A. W. Quart. Journ. Exper. Physiol., 1915, viii, 347.
(6) FRASER, L. M. This Journal, 1925, lxxii, 119.
(7) CLARK, W. M. The determination of hydrogen ions. Baltimore, 1923.
(8) BAUER, M. Arch. f. exper. Path. u. Pharm. 1926, cxii, cxiii, 205.
(9) MURLIN, J. R. AND H. A. MATTILL. This Journal, 1923, lxiv, 75.
(10) GROSS, L. Journ. Path. and Bact., 1924, xxvii, 27.
(11) OSBORNE, T. B. AND A. J. WAKEMAN. Journ. Biol. Chem., 1919, xl, 383.

THE DETERMINATION OF THE AMOUNT OF HEMOGLOBIN PRESENT IN RAT FETUSES DURING DEVELOPMENT

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Received for publication October 22, 1927

It was found necessary during the author's experiments upon rat fetuses to determine the rate of hemoglobin development in this form (1), (2). The results secured by Williamson and Ets (3) show that the amount of hemoglobin falls perceptibly between the twentieth and the sixtieth days of post-natal development. They further indicate that the amount of hemoglobin present at birth is probably higher than their ten-day figures would indicate. Our own results place the amount of hemoglobin at birth slightly lower than the figure given by them. It is to be admitted, however, that our results must of necessity be more of an approximation than an absolute quantitative measure of the amount of hemoglobin present. This is due to the necessity of working with exceedingly small amounts of blood and also to the comparatively few cases which we were able to use. The results of further determinations may change the quantitative but not the comparative value.

The difficulty in obtaining results which are at all uniform when dealing with materials which are so highly variable in size relationships as embryos, will at once be appreciated by anyone who has had to use similar material.

It should be noted that the mean is more than 72 times the probable error. The probable error can be shown to be much less than the assigned value if it be calculated on the basis of a weighted mean. If the materials and quantities be considered it is rather remarkable that the error is so small.

The obtaining of blood from rat fetuses in sufficient quantity for hemoglobin estimation was the first problem to be presented. Pregnant females, selected and grouped according to the age of the fetuses as determined by palpation, were operated upon under light ether anesthesia. The horns of the uterus were slit in such a way as not to injure the placenta. The fetus was removed with the fetal membranes intact and the placenta attached. Immediately upon removal from the uterus, the fetuses were placed in warm physiological saline until the determination sample could be taken. The amnion was removed just before the fetus

was taken from the saline, total length and crown-rump measurements were made which were checked against the Wistar tables in determining age groupings (4).

The fetuses which were sixteen days or above in the gestation period present no difficulty in obtaining the necessary amount of blood. In animals younger than this, however, there is such a small available blood content that it was difficult to secure even the few drops necessary for a determination. Under the conditions of removal and subsequent keeping for a short period in saline, the fetal blood apparently flows toward the placenta. It was only after the placenta accidently was lifted above the plane of the embryo that the blood gravitated toward the fetus and could be collected from the neck vessels. In all cases the blood for determinations was secured by decapitating the animal and securing the blood from

TABLE 1

AGE GESTATION	NUMBER OF CASES	DETERMINATIONS	HEMOGLOBIN*	VALUE OF MEAN
days			*per cent*	*grams per 100 cc.*
12–13	11	55	30	5.12 ±0.025
14	14	70	32	5.45 ±0.083
15	16	80	37	6.34 ±0.071
16	7	35	55	9.36 ±0.025
17	22	110	56	9.40 ±0.057
18	10	50	45	7.69 ±0.029
20	6	30	44	7.52 ±0.074
21	20	100	54	9.22 ±0.070
Newborn	7	35	65	11.05 ±0.227
Totals.....	102	560	Mean for series	7.9314±0 11

* 16.92 grams = 100 per cent.

the vessels of the neck. While a slight amount of other body fluids probably is collected by this method, it is improbable that this is of sufficient quantity to vitiate the comparative result.

The determination of hemoglobin was carried out according to the Newcomer method and the percentages are based on Williamson's normal value for the human (16.92 grams per 100 cc. = 100 per cent). Five determinations were made of each sample and these were averaged before adding the total number of individual cases which were also averaged. The diluting fluid was 1 per cent hydrochloric acid, the dilution being 1:502, this solution being corrected for the elapsed time between the addition of the hydrochloric acid and the hemoglobin determination. Since the standard Bausch and Lomb colorimeters are not adapted to measuring some of the low hemoglobin contents with which we had to deal, it was

necessary for us to use the 20 mm. microcolorimeter of the Duboscq type. This instrument was standardised against readings of the same solutions made with the Newcomer glass filter standard against known solutions in the 40 mm. standard colorimeter. Our method of standardisation showed that the Newcomer method can be applied to the microcolorimeter with the readings running as low as 20 to 25 per cent hemoglobin.

Traces of hemoglobin could undoubtedly be found much earlier than the lowest figure given in the above table. To ensure a qualitative test particularly at early stages would be possible particularly with microhistological methods but unfortunately these would give no idea of the quantity present.

Our results show that hemoglobin is present in a measurable amount before the 14th day of the gestation period in the rat. That is to say, the development of hemoglobin in measurable amounts begins during the later part of the second third of gestation. From this point, there is a significant rise from the 14th day to the 17th day, the most abrupt portion of the curve coming in the period between the 15th to the 16th days. There is a fall in the amount of hemoglobin on the 18th day of the gestation period with a succeeding rise on the final two days of gestation. The newborn rats show a slight increase in hemoglobin quantity over the fetus of only a few hours before. This increase may or may not be significant, the number of cases being too small to say definitely.

SUMMARY

1. The amount of hemoglobin present in rat fetuses has been measured at different periods of development.

2. The quantity of hemoglobin shows a gradual increase during the later part of the second third of the gestation period.

3. An extremely rapid rise occurs in hemoglobin content during the early part of the third period of gestation. This is followed by a slight drop and by a recovery which shows an increasing amount of hemoglobin present shortly after birth.

BIBLIOGRAPHY

(1) Nicholas, J. S. Anat. Rec., 1925, xxxi, 385.
(2) Nicholas, J. S. Proc. Soc. Exper. Biol. and Med., xxiii, 436.
(3) Williamson, C. S. and H. N. Ets. This Journal, 1926, lxxvii, 480.
(4) Donaldson, H. H. The rat. 2nd ed., Philadelphia, 1924.

THE GASEOUS METABOLISM OF THE DOG'S KIDNEY

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Received for publication October 15, 1927

Among experiments regarded as indirect evidence supporting views of secretory function of renal tubules are those which deal with the oxygen utilization of the kidney during the action of certain diuretics. It was pointed out by Loewi (1902) that according to the hypothesis of glomerular filtration and selective reabsorption, elimination of large volumes of dilute urine should be associated with less oxygen consumption than elimination of smaller volumes of a more concentrated urine. Barcroft and Brodie (1905) found that diuresis, instituted by urea or sodium sulphate, was always accompanied by an increased oxygen consumption. But since the urine during such a diuresis was more dilute than when oxygen consumption was less, they believed their experiments lent no support to Loewi's suggestion.

A few years later, following the work of Schlayer, Barcroft and Straub (1910) compared the oxygen consumption of the kidney during diuresis induced by Ringer's or sodium chloride solutions, with that following injection of urea, caffeine, sodium sulphate and phloridzin. They "regarded it as a fair assumption that if urine appears without increased oxidation in the kidney itself the mechanism which accounts for its appearance is a purely physical one, whereas, if there is a well marked increase in the metabolism of the kidney a secretory process is called into play." Their experiments led them to the belief that the diuresis following injections of Ringer's solution or sodium chloride was the result of alterations in the composition of the blood, leading to increased glomerular filtration, while that following urea, etc., was brought about by increased secretory activity of the tubule cells.

In similar experiments Bainbridge and Evans (1914) found no change in oxygen consumption accompanying alterations in rate of blood flow, while a marked increase followed injection of sodium sulphate. Knowlton and Silverman (1918) likewise detected no variation in metabolism with the changes in renal blood flow which followed the injection of pituitrin; urea or sodium sulphate, however, uniformly led to an increase. Tamura and Miwa (1919), on the contrary, could not detect any appreciable change in oxygen consumption during diuresis produced by caffeine, sodium sul-

phate or sodium chloride. Diuretin did increase the oxygen consumption; this they attributed to the sodium salicylate present. Tamura, Watanabe and Kabwiaki (1926) found the oxygen consumption of rabbit kidneys remarkably constant in different animals in spite of considerable variations in flow occurring spontaneously or following hemorrhage. Tashiro and Abe (1922) believed that a small dose of caffeine augmented the oxygen consumption of the kidney, while a large dose decreased it. Tamura (1926) and his associates on the other hand found the administration of caffeine without effect on the oxygen consumed by the kidney in rabbits, cats and dogs.

Since Barcroft's work direct proof of reabsorption has been obtained and the basis for belief that the most important force concerned in glomerular elimination is blood pressure has been strengthened. Greater justification exists now than then for belief that filtration and absorption may explain urine formation. This makes it desirable to subject the indirect evidence for secretion to further scrutiny.

We therefore undertook to repeat Barcroft's experiments and to compare the apparent metabolism of the kidney after injection of caffeine, urea or sulphate with that under other conditions. Certain differences in our experiments and those of our predecessors should be pointed out. We have had an excreting kidney at the beginning of each experiment. Attempts were made to vary the degree of diuresis without violent abnormalities in the degree of dilution of blood. Impressed with the rapidity of variations in renal blood flow and the influence of such changes on the calculation of oxygen consumption, we have taken pains to synchronize measurement of flow and collection of both arterial and venous samples.

Methods. Dogs were anesthetized with sodium pheno-barbital, 0.120 to 0.150 gram per kilo, and kept warm by an electric pad. Cannulae were inserted in trachea, left carotid for recording blood pressure, right carotid for collection of arterial blood samples, and a jugular vein for making injections. The gastro-intestinal tract was removed after ligature of its vessels. The right kidney was excised. The aorta was tied below the renal arteries. A cannula for collecting renal venous blood was placed in the cava below the left renal vein, and a loose ligature placed about it above the renal vein, by which blood flow was obstructed during the collection of samples. All visible veins emptying into the cava between the cannula and loose ligature except left renal were tied. The completeness of this procedure was confirmed at the end of each experiment by dissection or by injection. A cannula in the ureter was connected with a drop recorder and arranged for collection of urine. Artificial respiration was usually used throughout the experiment in order to assure more uniform oxygenation of the arterial blood.

Blood flow was determined by the method of Barcroft and Brodie, using

a 5 cc. pipette. Since the same pipette was used for collecting the venous blood sample, an estimation of flow was made during the collection as well as before and after. The blood in the 2 inches at either end of the pipette, which had had contact with air, was discarded. Arterial blood was collected in a 5 cc. syringe. A few cubic centimeters of blood were allowed to escape from both carotid and cava cannulae before collecting samples. Blood was immediately transferred to centrifuge tubes under oil. Clotting was prevented by oxalate. Great care was exercised to assure synchronism in measurement of blood flow and collection of blood samples. Blood volume was restored by injection of heparinized blood from another animal after collecting each set of samples. Samples were not taken until blood pressure, blood flow and urine were reasonably steady.

The O_2 and CO_2 content of the blood samples were determined in the constant volume apparatus of Van Slyke and Neill (1924). The analyses were usually run in duplicate. Duplicate analyses checked within 0.5 volume per cent with but few exceptions.

We have estimated the apparent metabolism of the kidney under a considerable range of blood flow and urine formation, and have compared the estimations following injection of urea, sodium sulphate and certain drugs with these. This "control" group includes thirty estimations on nine animals. Blood flow (and usually urine) was increased by injection of heparinized blood from another animal, hypertonic sodium chloride, physiological salt solution, 6 per cent gum acacia solution and hypertonic Ringer's solution. A decrease in renal blood flow followed the withdrawal of blood, injection of adrenalin, and circulatory failure. Estimations of the apparent metabolism of the kidney varied from 0.017 to 0.113 cc. per gram per minute; thirteen of the thirty lay between 0.040 and 0.055 cc. per gram per minute. Blood flow ranged from 0.42 to 5.3 cc. per gram per minute: at the time of thirteen of the collections it was from 1.98 to 3.02 cc. per gram per minute. Urine flow varied from 0.00 to 1.57 cc. per minute for periods of several minutes before samples were collected. The higher values for apparent metabolism were usually obtained when the blood flow was more rapid. No single experiment, however, exhibited a relation between rates of oxygen consumption and rates of urine flow which is convincing of a causal relation between the two, either in the sense of secretion or in the sense of active water reabsorption by an energy consuming process. Nor did any single experiment show a uniform relation between increase of blood flow and increase of oxygen consumption. But when the evidence of all the control observations is considered, a fair degree of correspondence is shown between blood flow and oxygen consumption. In figure 1 the "control" points derived from each experiment are connected by light lines, while the heavy line is plotted from averages of the estimations for each cubic centimeter per gram per minute increment in blood flow.

Calculations of the apparent metabolism after injection of caffeine, urea or sodium sulphate did not yield any figures higher than were obtained in the control group. In general the oxygen consumption agreed fairly well with that found at a similar blood flow at other times in the experiments. The same is true of estimations following the exhibition of pituitrin, morphine, ephedrine and, in one experiment, phloridzin. When the estimations of oxygen consumption following injections of these sub-

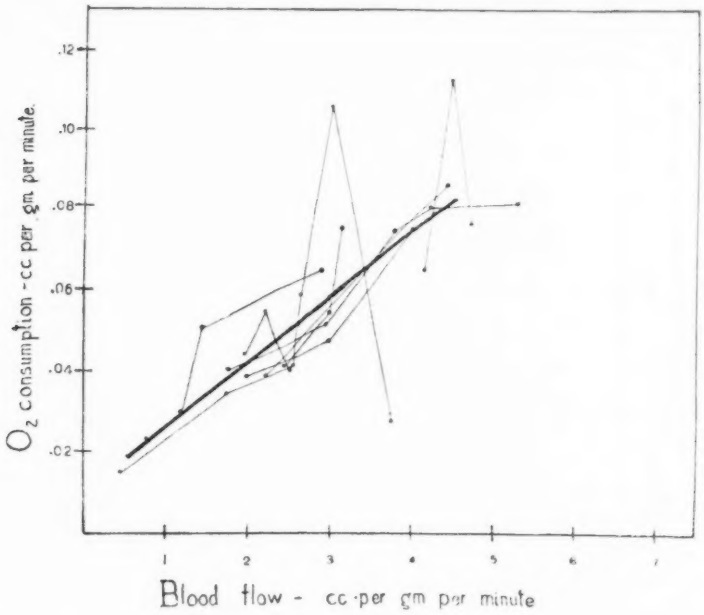

Fig. 1. Relation of blood flow and oxygen consumption in control estimations. Those derived from each experiment are connected by light lines. The heavy line was drawn from group averages, each group including all estimations within a cubic centimeter per minute increase in blood flow.

stances are inserted on the control chart, they are distributed in such a way that if included in the calculations of the average curve, its character is not significantly altered. In figure 2 all the estimations from nine experiments were used in drawing the curve; for the sake of clearness the individual control points shown in figure 1 are not plotted.

Calculations of the amount of carbon dioxide added to the blood passing through the kidney showed wide variations. However, a distinct drift toward larger additions per gram of kidney per minute at high blood flow

seems evident (fig. 3). The relation of carbon dioxide production to oxygen consumption yields figures for respiratory quotients which we are unable to interpret at the present time. Similar difficulties have been encountered by others in comparisons of blood gases from samples collected over brief periods. Part of the data from four of the experiments are presented in table 1, in order to indicate the variations in blood pressure and blood concentration which accompany changes in flow. Because of

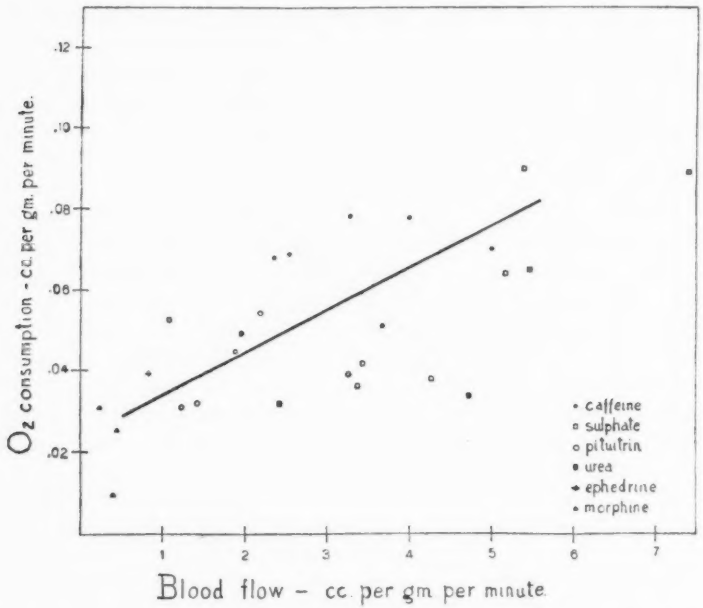

Fig. 2. Relation of blood flow and oxygen consumption after the administration of drugs. All estimations were used in plotting the curve; for clearness control points shown in figure 1 are not plotted.

the limitations of space similar data from the other experiments as well as all figures for carbon dioxide have been omitted.

Analyses of the urine collected during the different periods of these experiments failed to aid in correlating the oxygen consumption of the kidney with the concentration or composition of the urine. This is consonant with the findings of others, for in order to calculate even the minimum work required in overcoming osmotic resistance the concentration of every dissolved substance in blood (or glomerular fluid) and urine must be known.

Since the magnitude of the blood flow is the dominant factor in the calculation of apparent metabolism in these experiments, it was necessary to see whether the substances of especial interest, caffeine and sodium sulphate altered the apparent metabolism of the kidney when blood flow was kept constant. For this purpose the kidneys of two animals were perfused with a Richards-Drinker (1915) pump, which enables blood flow to be kept constant.

The animal was prepared as previously described, except that the pedicle of the right kidney was tied with mass ligatures and not removed.

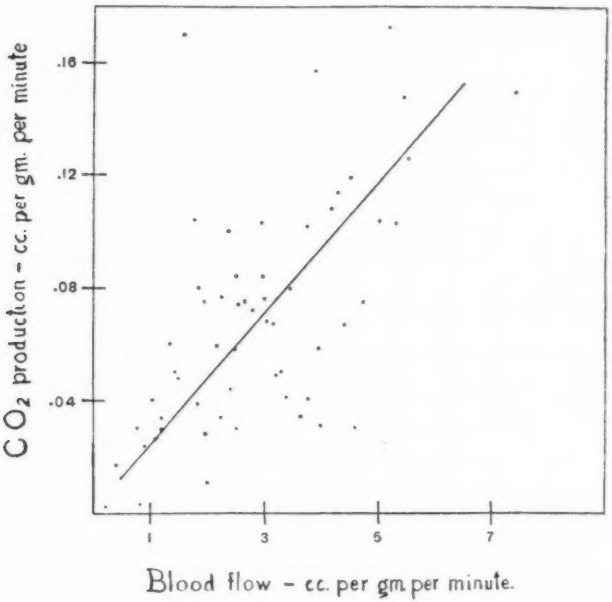

Fig. 3. Relation of blood flow and CO_2 production

Bleeding was controlled by cautery. Blood was prevented from clotting by injection of 0.1 gram heparin per kilo. A branched cannula was inserted into the stump of the superior mesenteric artery, and another into the left renal artery close to the aorta: the side arms of the cannulae were connected, so that the kidney received blood by way of the superior mesenteric artery. The length of time the kidney was without a supply of blood was one to two minutes. The pump was connected to the other branch of the renal artery cannula with a bypass to the inferior cava.

The pump was adjusted to provide a blood flow adequate for moderate

secretion of urine. In the first experiment, the apparent metabolism was the same in two control estimations four minutes apart, and was not in-

TABLE I

EXPERIMENT NUMBER	TIME	BLOOD PRESSURE	BLOOD FLOW	URINE	O_2 CONTENT OF BLOOD		O_2 CONSUMPTION	REMARKS
					Arterial	Venous		
		mm. Hg	*cc./ gm./ min.*	*cc./ min.*	*vol. per cent*	*vol. per cent*	*cc./ gm./ min.*	
8	2:59	102	2.99	0.10	18.14	16.56	0.048	End of preparation
	3:19	130	4.00	0.56	19.81	17.92	0.075	After 25 cc. blood+25 cc. 0.9 per cent NaCl
	3:32	130	5.00	1.03	19.49	18.09	0.070	After 80 mgm. caffeine
	3:50	136	5.40	3.60	18.06	16.40	0.090	After 10 cc. 20 per cent Na_2SO_4
	4:08	160	5.49	2.10	18.73	17.51	0.067	After 1 cc. pituitrin
	4:28	114	2.00	0.13	18.12	16.04	0.041	0.25 cc. 1:10,000 adrenalin at 4:16
	4:44	88	0.40	0.02	18.31	15.87	0.009	After 40 mgm. morphine
10	2:37	64	0.78	0.03	18.57	15.34	0.025	End of preparation
	2:52	108	1.47	0.06	18.61	14.98	0.053	After 50 cc. blood+50 cc. 0.9 per cent NaCl
	3:20	130	2.86	0.49	19.48	17.21	0.065	After 100 cc. blood+115 cc. 0.9 per cent NaCl
	3:34	86	3.28	0.58	20.05	17.66	0.078	After 80 mgm. caffeine
	3:46	86	3.43	2.20	18.16	16.94	0.042	After 20 cc. 20 per cent Na_2SO_4
	4:03	100	3.28	2.23	19.46	18.27	0.039	After 1 cc. pituitrin
	4:21	88	1.07	0.02	19.14	16.25	0.031	After adrenalin
	4:30	68	0.91	0.00	19.83	16.81	0.027	After 80 mgm. morphine sulphate
13	12:41	132	3.78	0.33	17.97	16.02	0.074	End of preparation
	12:58	142	4 25	0.44	18.39	16.51	0.080	After 50 cc. blood
	1:11	162	5.30	1.57	16.86	15.33	0.082	After 10 cc. hypertonic Ringer's
	1:33	150	4.70	2.06	16.04	15.31	0.034	After 2 gm. urea
	1:47	134	3.99	2.87	17.95	16.00	0.078	After 80 mgm. caffeine
	1:54	156	5.15	4.46	16.08	14.83	0.064	After 20 cc. 20 per cent Na_2SO_4
18	12:43	104	4.15	0.13	16.24	14.67	0.065	End of preparation
	1:07	136	4.69	1.56	15.82	14.28	0.072	After 10 cc. 5 per cent NaCl
	1:21	142	7.38	4.40	14.57	14.20	*·089	After 20 cc. 20 per cent Na_2SO_4
	1:55	83	4.48	0.75	17.00	14.47	0.113	End sulphate diuresis
	2:30	85	2.35	1.25	13.92	11.01	0.069	After 60 mgm. caffeine
	2:41	94	2.52	0.12	13.91	11.16	0.065	After 10 mgm. ephedrine

* Corrected for concentration of venous blood.

creased by injection of caffeine, although perfusion pressure fell and rate of urine flow increased. Splitting of one of the rubber connections necessitated stopping the pump for repair. During this period the kidney was

supplied with blood via the superior mesenteric cannula. When the pump was started again some 50 minutes later, the apparent metabolism at the same blood flow had fallen about 20 per cent. Sodium sulphate was followed by a slight fall in perfusion pressure and diuresis, without change in oxygen consumption. In the second experiment the pump was adjusted to deliver approximately the same volume as in the first experiment. This flow, however, seemed somewhat excessive for the particular kidney, as evidenced by the profuse diuresis and abnormal height of perfusion pres-

TABLE 2

EXPERIMENT NUMBER	TIME	SYSTEMIC BLOOD PRESSURE	PERFUSION PRESSURE	BLOOD FLOW	URINE	O_2 CONTENT		O_2 CONSUMPTION	REMARKS
						Arterial	Venous		
		mm. Hg	*mm. Hg*	*cc./ gm./ min.*	*drops/ min.*	*vol. per cent*	*vol. per cent*	*cc./ gm./ min.*	
P 1	1:49	48	140	2.20	5	15.30	12.67	0.058	Control
	1:53	40	140	2.20	3	15.46	12.81	0.058	Control
	2:00	36	70–120	2.08	6*	15.45	13.04	0.050	After 40 mgm. caffeine in pump reservoir
	2:50	27	111	2.05	3	16.06	13.88	0.045	After repair of pump
	3:08	32	96	2.03	29	13.17	11.14	0.041	After 20 cc. 20 per cent Na_2SO_4 in pump reservoir
	3:14	47	144	2.08	35	13.31	11.36	0.041	After 1 cc. pituitrin in pump reservoir
P 2	1:29	98	200	2.00	17	16.52	13.08	0.069	Control
	1:40	92	about 230	1.93	14	17.60	14.45	0.061	Control
	1:52	38	about 230	2.04	21	16.86	13.83	0.062	After 20 mgm. caffeine in pump reservoir
	2:00	54	about 240	2.06	26	16.97	14.59	0.049	Late caffeine?
	2:12	100	about 230	2.00	33	15.29	12.29	0.060	After 10 cc. 20 per cent Na_2SO_4 by vein

* Drop recorder not recording all drops.

sure. The apparent metabolism was greater throughout than that usually found with the flow used. Caffeine again was not followed by any increase in oxygen consumption, but rather by a decrease. This has been attributed to a poisonous action by Barcroft and Straub. Sodium sulphate did not increase the apparent oxygen consumption above the control level (table 2).

DISCUSSION. Our experiments are subject to the same major criticisms as are those of Barcroft and Straub. The validity of calculating metabolism from analyses of isolated samples of blood is questionable. Small

errors in measurement of blood flow exert a preponderant effect on the calculated metabolism. The oxygen disappearing from a small quantity of blood passing through an organ in a brief time may not be the same as the amount actually entering into the metabolism of the tissue during that period.

Mindful of such limitations, we have been unable, under the conditions of these experiments, to detect any influence of caffeine, urea, or sodium sulphate on the oxygen metabolism of the kidney which would encourage the belief in the tubular secretion of these substances as opposed to others.

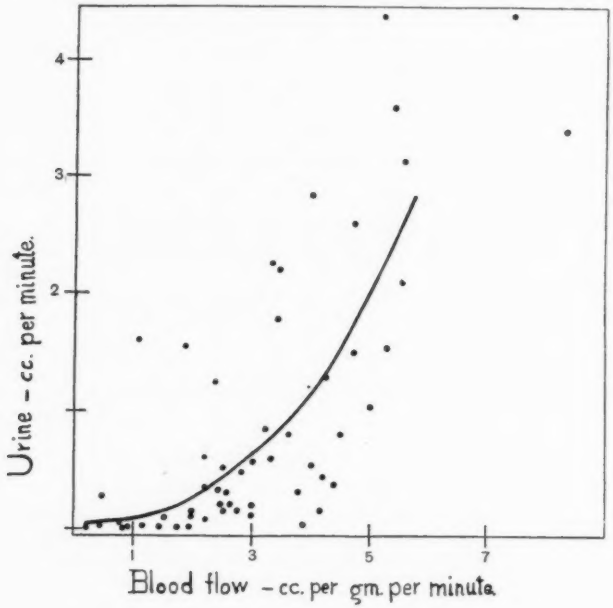

Fig. 4. Relation of blood flow and urine formation

We have found that the coefficient of oxygen utilization by the kidney is usually smaller with large volume flow of blood than it is with small volume flow. But the relation between decrease in coefficient and increase in blood flow is usually such that the calculated amount of oxygen consumed by the kidney is greater at high blood flows. The reason for this is not clear. It seems unlikely that a mere increase in blood flow should increase the metabolism of kidney cells. Such a conclusion would be at variance with the work of Barcroft and Müller (1912) on the salivary gland and of Rapport and Katz (1927) on isolated striped muscle. Verzár (1912),

however, has reported that the less the circulation through a muscle, the less the consumption of oxygen. Nakamura (1921) could not confirm Verzár's work and has criticised his technique.

Evidence has been presented that the number of glomeruli through which blood flows is not constant, and that even with flows of over 3 cc. per gram per minute in a rabbit all of the glomeruli may not be receiving blood (Hayman and Starr, 1925). In general, however, there was a direct relation between the volume flow and number of open glomeruli. The relation of glomerular flow to the blood supply of the corresponding tubule and the finer details of tubular capillary anastamoses have not been definitely described. Recent studies (Morison, 1926) indicate that the chief supply of a proximal and distal convoluted tubule is derived from its own glomerulus. Thus an increase in volume flow of blood may involve not only the perfusion of more glomeruli, but also of a greater amount of tubular tissue where it is probable the largest part of the oxygen consumption takes place. It has been shown that increment of blood pressure, unaccompanied by increment in volume of blood flow increases urine (Richards and Plant, 1917); yet increase in volume flow of blood must be a secondary diuretic factor since it increases the rate of renewal of fluid in contact with the filtering membrane (fig. 4). If the elaboration of the glomerular fluid is an energy-consuming process, increase in amount may entail increased work on the part of the tubules in its elaboration, the relation between the amount of work and the final volume of urine depending upon the composition of the two fluids, the condition of the tubule cells and undoubtedly on other unrecognized factors.

CONCLUSIONS

The apparent oxygen metabolism of the kidney was estimated in nine dogs by the method of Barcroft and Brodie. The oxygen consumption varied from 0.009 to 0.113 cc. per gram per minute, the higher figures usually accompanying the greater blood flow and more profuse diuresis. The injection of urea, caffeine, sodium sulphate, or certain drugs was not folowed by any characteristic effect on apparent oxygen metabolism.

In two kidneys perfused with constant blood flow diuresis induced by caffeine or sodium sulphate was not accompanied by an increase in the amount of oxygen disappearing from the blood per minute.

BIBLIOGRAPHY

BAINBRIDGE, F. A. AND C. L. EVANS. 1914. Journ. Physiol., xlviii, 278.
BARCROFT, J. AND T. G. BRODIE. 1905. Journ. Physiol., xxxii, 18; xxxiii, 52.
BARCROFT, J. AND F. MÜLLER. 1912. Journ. Physiol., xliv, 259.
BARCROFT, J. AND H. STRAUB. 1910. Journ. Physiol., xli, 145.
HAYMAN, J. M., JR. AND I. STARR, JR. 1925. Journ. Exper. Med., xliii, 641.

KNOWLTON, F. P. AND A. C. SILVERMAN. 1918. This Journal, xlvii, 1.
LOEWI, O. 1902. Arch. exper. Path. u. Pharm., xlviii, 410.
MORISON, D. M. 1926. Amer. Journ. Anat., xxxvii, 53.
NAKAMURA, H. 1921. Journ. Physiol., lv, 100.
RAPPORT, D. AND L. N. KATZ. 1927. This Journal, lxxx, 185.
RICHARDS, A. N. AND C. K. DRINKER. 1915. Journ. Pharm. Exper. Therap., vii, 467.
RICHARDS, A. N. AND O.H. PLANT. 1917. This Journal, xlii, 592.
TAMURA, K. AND M. MIWA. 1919. Mitt. d. med. Fakultat. d. Kaiserl. Univer. z. Tokyo, xxiii, 319.
TAMURA, K., S. WATANABE AND H. KABURAKI. 1926. Proc. Imperial Acad. Tokyo, ii, 431.
TAMURA, K., S. WATANABE, Y. T. SHAH, S. KOMATSUBARA AND G. KIHARA. 1926. Proc. Imperial Acad. Tokyo, ii, 434.
TASHIRO, K. AND H. ABE. 1922. Tohoku Journ. Exper. Med., iii, 142.
VAN SLYKE, D. D. AND J. M. NEILL. 1924. Journ. Biol. Chem., lxi, 523.
VERZÁR, F. 1912. Journ. Physiol., xliv, 243.

AN ATTEMPT TO STRIKE A BALANCE OF CARBOHYDRATE METABOLISM AT THE TIME OF RAPID CHANGES IN BLOOD SUGAR AND LIVER GLYCOGEN

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Received for publication October 17, 1927

Endogenous hyperglycemias fall into four groups: 1, pharmacological, e.g., those induced by narcotics and anesthetics; 2, asphyxial, now considered to be due to anoxemia (Kellaway, 1920); 3, hormone, e.g., that induced through the activity of the adrenal glands; 4, nervous, e.g., that induced by stimulation of the splanchnic nerves, or the piqûre center. Since these hyperglycemias develop with such rapidity it seemed worth while in certain of them to make a quantitative study of the shift of glycogen into sugar and the reverse, in an endeavour to strike a balance between the carbohydrate lost in one organ, e.g., the liver, and that gained elsewhere, e.g., the blood stream.

Although the Cori's (1923) used the "abdominal window" method for the taking of successive liver samples in rabbits without causing hemorrhage, or involving the use of anesthetics, the "decapitate cat" as a preparation for this purpose has a distinct advantage, in that, with the medulla gone, carbohydrate metabolism cannot be affected by those influences which, in a normal animal, might act on the glycogenic center. All of our cats were starved for twenty-four hours previous to experimentation, so that the complication of alimentary absorption was avoided.

The percentage of sugar in the blood was estimated by the Shaffer-Hartmann method, with the modification that flasks having rubber stoppers fitted with Bunsen valves were used to prevent reoxidation during cooling. Blood was always taken from the carotid artery.

To obtain liver samples a median abdominal incision was made, a loop of $\frac{1}{4}$ inch tape passed around a small portion of liver tissue (weighing from 1 to 2 grams), and the latter immediately removed and dropped into hot potassium hydroxide, to digest in a boiling water-bath for three hours. The tape ligature was tied just tight enough to prevent hemorrhage without lacerating the tissue: there was less likelihood of toxic absorption from the site of such a wound than there would be were the sample cut off with hot scissors only. The liver sample was obtained within three minutes of the blood sample, and the abdomen was immediately closed to prevent

cooling. Glycogen from the piece of liver was isolated and washed by Pflüger's method (1905) and the glucose content after acid hydrolysis estimated by the Shaffer-Hartmann method as outlined above.

The justification of calculating the concentration of glycogen in the whole liver, from that found in any one lobe, may be questioned on account of the work of Dowler and Mottram (1918). These workers claimed that there was great irregularity in the concentration of glycogen in various lobes, variations sometimes amounting to 63 per cent of the highest concentration. However, Macleod and Pearce (1911) claim that the distribution in different portions of the liver is almost exactly uniform in cats and dogs. Working on cats starved for 24 hours we found in two experiments that the amount of glycogen was practically the same in all parts of the liver: moreover, at the close of nearly all experiments we took two or three samples from as many different sites in the liver, and the glycogen content of these never varied from one another by more than 10 per cent, and usually by considerably less.

The removal of pieces of liver did not have any marked detrimental effect on the blood sugar level. In no instance did the blood sugar fall below normal or remain at an unusually high level. This is interesting in view of the work by Mann (1925) in which complete extirpation resulted in a steady fall in the level of blood sugar. In our experiments, despite the initial anesthesia, the blood sugar fell to between 0.1 per cent and 0.15 per cent in from four to eight hours, and remained at that concentration when not interfered with. Thus, the liver gave no evidence of having lost its property of maintaining the blood sugar at a constant level. This is hardly to be wondered at since even in the longest experiments there was still two-thirds of the liver left in the animal at the end of the experiment.

In order to compare the amounts of these two forms of carbohydrate present in liver and blood, it was necessary to calculate their concentration in milligrams from the percentages already obtained by the Shaffer-Hartmann method. The total volume of blood in the body was taken as being equivalent to one-thirteenth of the weight of the intact animal. At the close of the experiment the remnant of the liver was freed from the gall bladder, bile duct and as much connective tissue as possible, and weighed. As the weight of each sample taken during the period of the observation was known, the weight of the liver at the time each sample was removed, could easily be calculated. It was found that between two and three grams of tissue was rendered bloodless by each ligature, this was deducted from the estimated functioning amount of liver from which the succeeding sample was taken.

Anesthesia. In all decapitate preparations ether was administered prior to the operation, and as the operation was completed in 7 to 15 minutes after commencement of anesthesia, the animal was never subjected to ether for any great length of time.

The usual hyperglycemia resulted, while the liver glycogen immediately after decapitation was invariably low (fig. 1). During the succeeding hours the liver glycogen gradually increased in concentration up to a maximum which varied with the store of carbohydrate in the animal, and was reached in from three to eight hours after the operation. At the same time the blood sugar fell, but not to a corresponding degree. These glycogen maxima varied according to the nutritional condition of the cat. For example, in the case of two cats of approximately the same weight, one (no. 18), which had large deposits of fat throughout the body, reached a glycogen maximum of 7.3 per cent, while the other (no. 11), a thin cat, reached a maximum of only 0.8 per cent. When the blood sugar had fallen to approximately 0.1 per cent, it remained practically stationary, but the glycogen content of the liver fell continuously once its maximum had been reached. The beginning of the fall in liver glycogen was sometimes coincident with the establishment of a steady level in blood sugar but in several cases both liver glycogen and the blood sugar were falling at the same time.

Although none of our experiments included a determination of the liver glycogen prior to the onset of anesthesia, it was obvious from the analyses performed immediately after decapitation, that very considerable glycogenolysis had been going on in the liver and that large amounts of sugar had been liberated into the blood stream. It might be thought that a part of the sugar so liberated would pass to the muscles, because the tension of sugar in them would be lower than in the blood, and the sugar would then be stored as glycogen. If this were true, the glycogen, according to the work of Soskin (1927) would, so to speak, be firmly locked up in the muscles only to be liberated in the course of muscular exercise. This would lead one to infer that the restoration of glycogen in the liver in the post-operative stage would be limited and would have to be considerably less in amount than that which would come from the extra sugar free in the blood stream. But analysis of six curves showing the after-effects of anesthesia and decapitation demonstrates that the rise in concentration of hepatic glycogen is *much greater* than can be accounted for by the fall in blood sugar, even if all the extra blood sugar went to the liver, and none to the muscles.

Whatever be the cause of this period of glycogenesis, the site and nature of the substance from which glycogen is formed remains undetected. The process is apparently independent of the level of glycogen in the liver after anesthesia, because glycogenesis invariably proceeds, be the liver rich or poor in glycogen.

A further examination of the curves shows that after the blood sugar has reached a steady level, even though this be considerably above normal, the liver glycogen sooner or later begins to fall, presumably to provide the necessary energy for basal metabolism and maintain a constant blood

sugar level. For example, in one (cat 4) the liver glycogen began to fall when the blood sugar was still 0.267 per cent, while in cat 3 the liver glycogen did not begin to fall until after the blood sugar had been at 0.112 per cent for some time. From this it again appears that there can be no direct quantitative relationship between the changes in liver glycogen and those in blood sugar.

Langfeldt (1920), writing on the control of the blood sugar level, offers the theory that while blood flow and temperature remain constant, the glucose discharged is:

a. Burned in metabolic processes.

b. Stored in the cells of muscles and other tissues as glycogen.

c. Returned to the liver and resynthesized to glycogen, depending on the presence of the pancreatic hormone and the carbohydrates and proteins of food.

TABLE 1

Cat 3. Weight 4 kilos. Anesthesia and asphyxia

	HOURS AFTER DECAPITATION	BLOOD SUGAR		LIVER GLYCOGEN			TOTAL GLUCOSE
		Per cent	Grams	Weight of liver	Per cent	Grams	
							grams
1	$\frac{1}{4}$	0.380	1.24	110	1.33	1.46	2.70
2	$2\frac{1}{2}$	0.239	0.775	$108\frac{1}{4}$	1.99	2.16	2.94
3	5	0.112	0.364	106	2.10	2.23	2.59
4	$6\frac{1}{2}$	0.113	0.366	$104\frac{3}{4}$	1.33	1.39	1.79
(Asphyxia for 4 minutes and aeration for 2 minutes immediately preceding sample 5)							
5	$6\frac{3}{4}$	0.339	1.10	$107\frac{1}{2}$	0.20	0.20	1.30
6	$7\frac{1}{4}$	0.271	0.879	$98\frac{1}{4}$	0.16	0.16	1.04

As there is no reason to believe that the metabolic rate of a decapitate cat, at constant temperature, changes from hour to hour, or that the rate of deposition of glycogen in resting muscles alters, we must conclude that once the maximum is reached the process of resynthesis of glycogen in the liver becomes considerably slowed up, or that the available stores of carbohydrate in the body are being diminished. It is hardly conceivable that in such relatively short experiments as ours, glycogenesis should be so markedly affected by failing food supplies. It may be objected that the glycogenic process has fallen to a lower level than that of glycogenolysis because of the cumulative detrimental effect of removing portions of the liver. This can hardly be true because later experiments (e.g., fig. 2 and table 3) showed that when no samples were taken from the liver until after the lapse of eight hours, the slowing up of glycogenesis occurred at approxi-

mately the same time as would have been expected had samples been taken from the beginning of the experiment; nor did the percentage of glycogen in the liver tend to rise any higher when opening of the abdomen and section of the liver was postponed for several hours. Impending death,

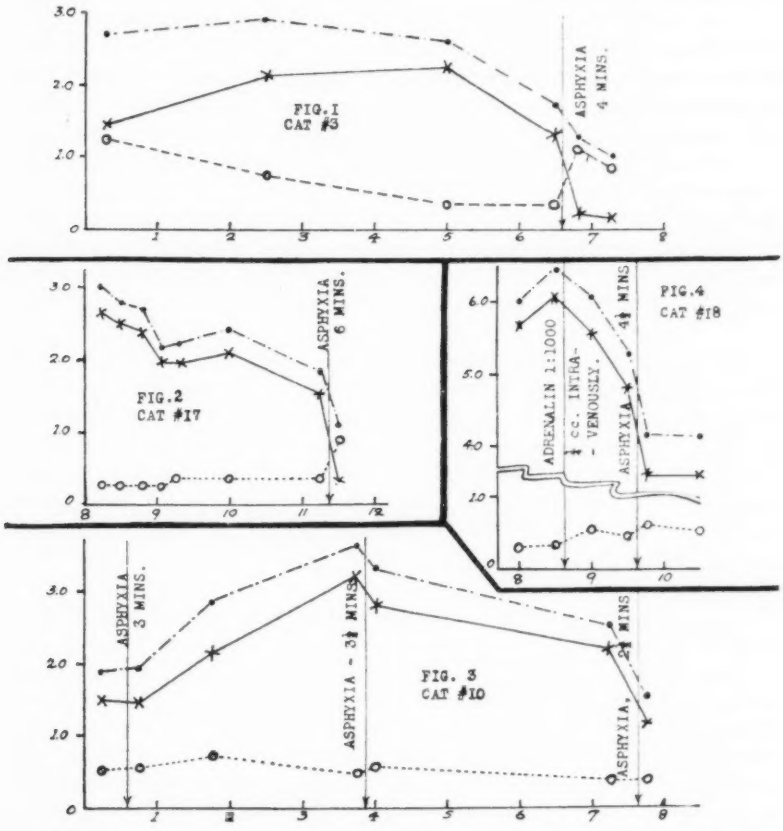

Fig. 1. Cat 3. Weight, 4 kilos. Liver, 110 grams. General contour of curves after decapitation and asphyxia.

Fig. 2. Cat 17. Weight, 2.83 kilos. Liver, 82.5 grams. Effect of asphyxia some time after decapitation.

Fig. 3. Cat 10. Weight, 3.3 kilos. Liver, 91 grams. Effect of asphyxia at different periods after decapitation.

Fig. 4. Cat 18. Weight, 2.8 kilos. Liver, 88 grams. Effect of adrenalin injection and asphyxia.

In each figure—Lowest curve represents blood sugar; middle curve represents liver glycogen; uppermost curve represents sum of blood sugar and liver glycogen. Ordinates—glucose in grams (*not* per cent). Abscissae—hours after decapitation.

though a natural, is hardly a justifiable objection, because nearly all the cats were in splendid condition at the close of the experiments, and had to be killed. Moreover, ante-mortem glycogenolysis is usually marked by a distinct rise in blood sugar concentration, and this did not occur in any of the fifteen cases observed.

In an endeavor to determine the cause for the post-operative rise in liver glycogen, it was decided to test the effect of anesthesia alone, and of delayed decapitation. Three such experiments convinced us that the intact animal kept under ether for as long as four hours showed no evidence of glycogenesis. On the contrary, the liver glycogen fell slightly in each case. For example, in one (cat 28), the liver glycogen ½ hour after commencing etherization was 1.49 per cent, at the end of 4 hours it was 1.05 per cent; the blood sugars at these times were, respectively, 0.316 and 0.270 per cent. The animal was then decapitated. The liver glycogen fell at once to 0.45 per cent and the blood sugar rose to 0.319 per cent. Three-quarters of an hour later the glycogen was 1.04 per cent and the blood sugar 0.296 per cent.

This shows that neither anesthesia nor our manipulation of the liver is responsible for the sudden post-operative glycogenolysis and the slower subsequent glycogenesis. It seems most probable that the immediate effect of decapitation is to stimulate the fibers from the piqûre center so that glycogenolysis occurs, but that in a very short time glycogenic processes in the liver begin, perhaps stimulated by the excess of sugar in the blood. It was observed in these experiments that in the intact animal ether prevents the deposition of glycogen in the liver although the blood sugar may be very high.

Asphyxia. Asphyxia is known to cause hyperglycemia not only when there is a plentiful supply of glycogen in the liver, but also when the percentage in that organ is low. This we found to be the case, and indeed asphyxia proved to be the most effective method at our disposal for producing hyperglycemia. Asphyxia was easily brought about by shutting off the artificial respiration until the heart became very irregular; this meant absolute anoxemia for from 3 to 6 minutes (usually 5 minutes) and during this interval the cat showed signs of sympathetic stimulation, such as standing up of the hair, and usually passed into convulsions of varying degrees of severity.

When asphyxia occurred soon after decapitation, while gluconeogenesis was increasing the hepatic concentration of glycogen hour by hour, it acted merely as a temporary set back to the synthetic process, and the lost carbohydrate was for the most part recoverable in the blood stream. In cat 10 (fig. 3) the glycogen remained unaltered although the blood sugar rose to 0.04 per cent.

When, however, asphyxia took place as the percentage of glycogen in the

liver was falling, glycogenolysis was rapid and marked, expecially in the first few minutes, after which it continued falling with varying degrees of rapidity, sometimes coming to a standstill, but never showing signs of recovery. In only three cases was this terminal fall in liver glycogen to any degree balanced by a rise in blood sugar; in twelve others very little of the lost glycogen could be recovered as sugar.

It is hardly likely that this loss can be accounted for by the muscular exertions during asphyxia, as these were of such short duration, and the liver samples were obtained within two minutes of the cessation of asphyxia; the transfer of glycogen from liver to muscle would presumably take longer than this. No respiratory movements took place. And yet, these terminal asphyxias usually brought the liver glycogen down to 0.5 per cent or less. As the blood sugar level rapidly fell to normal without a corresponding rise in liver glycogen, it is obvious that the liver was rapidly losing, or had already lost, its power of glycogen synthesis.

TABLE 2

Adrenalectomy followed (1) early and (2) late, by asphyxia

CAT NUMBER	DURATION OF ASPHYXIA	HOURS AFTER ADRENALECTOMY	CHANGES IN	
			Blood sugar	Liver glycogen
	minutes	*hours*	*per cent*	*per cent*
35	$3\frac{1}{2}$	$\frac{3}{4}$	−0.03	+0.77
36	4	$\frac{3}{4}$	unchanged	−0.47
8	5	1	+0.04	−0.60
7	4	$1\frac{1}{4}$	−0.02	−0.40
35	4	$3\frac{3}{4}$	+0.27	+0.75
6	5	$5\frac{1}{2}$	+0.20	−0.80
7	$3\frac{1}{4}$	$5\frac{3}{4}$	+0.13	−1.40

That the increased sugar in the blood does come from glycogenolysis, and that the source of this glycogen is hepatic and not muscular, seems beyond dispute from the work of Macleod (1913) and Soskin (1927) already discussed. Yet we found it impossible to balance these two forms of carbohydrate in four-fifths of the cases observed.

ASPHYXIA FOLLOWING BILATERAL ADRENALECTOMY. The senior author (Olmsted, 1926) had found that in decapitate cats typical asphyxial hyperglycemia did not occur after bilateral adrenalectomy. Upon repeating this work in order to compare the effect on liver glycogen with that on blood sugar, it was found that in certain cases the typical rise in blood sugar and fall in liver glycogen did occur. A tabulation of all the results showed that when asphyxia followed immediately after adrenalectomy there was no rise in blood sugar, a confirmation of the earlier results, but that when asphyxia was postponed until between two and six hours after

the operation, it produced the usual rise in blood sugar. The results are shown in table 2. The experiment on cat 35 brings out the point well. One-half hour after decapitation the blood sugar was 0.264 per cent, and the liver glycogen 5.00 per cent. The preparation was then asphyxiated for 3½ minutes and the blood sugar rose to 0.309 per cent and the liver glycogen fell to 3.70 per cent. Bilateral adrenalectomy was then performed, after which the blood sugar was 0.277 per cent and liver glycogen 3.15 per cent. One-half hour later the preparation was again asphyxiated for 3½ minutes. The blood sugar was found to be at a lower level than just after adrenalectomy, i.e., 0.251 per cent, and the liver glycogen higher, i.e., 3.92 per cent. In other words, the usual fall in blood sugar and rise in liver glycogen after decapitation was going on regardless of the asphyxia. The blood sugar continued to fall and 4½ hours after decapitation it was 0.120 per cent. The liver glycogen had already passed its maximum of 4.00 per cent and at this time was on the down grade, being 1.31 per cent. Another period of asphyxia at this point now caused the blood sugar to rise to 0.380 per cent and the liver glycogen followed suit, rising to 2.06 per cent.

These results can be explained on either of the following hypotheses: 1, the hyperglycemia of asphyxia is normally due to hypersecretion of adrenalin, and consequently is very slight or altogether absent in the absence of the adrenal glands, but that the body is able to adjust itself to the loss of the adrenals, so that the power of asphyxia to produce hyperglycemia becomes more and more marked in the adrenalectomized animal, as time goes on. This may be due to full excitability of the nerve path to the liver despite the absence of epinephrin; or 2, the adrenals really have little to do with the rise in blood sugar, and that the failure of the latter to respond to asphyxia immediately after adrenalectomy is due to post-operative shock.

ADRENALIN HYPERGLYCEMIA. It is generally agreed that the extent of the hyperglycemia brought on by adrenalin is dependent on the concentration of glycogen in the liver (Ritzmann, 1909). Even when the latter is extremely low, however, a certain amount of glycogenolysis and hyperglycemia still occurs (Bang, 1913, and others).

We were, therefore, disappointed at finding that in two separate experiments, the injection of ½ cc. and 1 cc. of 1:1000 adrenalin taken from separate sources, did not produce the typical effects. Probably after-effects of anesthesia and decapitation were still too pronounced to allow other effects to become evident. In the first cat the post-operative percentage of glycogen in the liver was extremely low (0.2 per cent) and even two hours after decapitation this had risen only 1 per cent. The adrenalin had not the slightest effect. The same applies to the second preparation. At the time the adrenalin was injected, the blood sugar was still at the high level of

0.34 per cent. This may have obscured the observation, though it would probably have been possible to force the blood sugar much higher by means of asphyxia.

It was therefore decided to test the action of adrenalin after ether hyperglycemia had subsided. Eight hours after decapitation, two blood and liver samples were taken with an interval of half an hour between each, and then 0.25 cc. of adrenalin (1:1000) was injected into the superior mesenteric vein. Incidentally, the cat was very fat and the liver contained 7 per cent glycogen. Within half an hour the blood sugar had risen from 0.183 to 0.290 per cent, but fell to 0.234, in the succeeding half-hour; the corresponding liver glycogens were 7.28, 6.87 and 6.35 per cent, a steady fall. When measured in milligrams of glucose, this loss of glycogen and increase in blood sugar do not balance eachother.

One hour later the cat was asphyxiated for $4\frac{3}{4}$ minutes. The blood sugar rose from 0.234 to 0.304 per cent in 15 minutes and then gradually subsided. At the same time the liver glycogen fell from 6.4 to 5.10 per cent and remained stationary at that level (see fig. 4).

In all the hyperglycemias studied thus far, it had been found impossible to strike a balance between the liver glycogen and the blood sugar. As has been pointed out, the total carbohydrate from these two sources rises, after decapitation, to a peak and then gradually declines. The contour of the curve is invariable. It was thought that by determining the free sugar and lactic acid content of the liver, simultaneously with the liver glycogen and blood sugar, that some of the missing carbohydrate might be found.

Method. On opening the abdomen, a somewhat larger piece of liver than usual (weighing 3 to 4 grams) was removed. It was immediately divided, one part dropped into hot potassium hydroxide for glycogen determination and the other into ice cold hydrochloric acid saturated with sodium chloride, and therein minced as finely as was possible. The second portion was left for 12 hours or more in the refrigerator, to allow extraction to proceed, and then $HgCl_2$ was added, to precipitate the proteins. After filtration, the mercury was removed by bubbling hydrogen sulphide through the filtrate, and after further filtration the sulphur was blown off with compressed air. This solution was neutralised and duplicate 5 cc. samples used for determining the concentration of free carbohydrate by the Shaffer-Hartmann method.

It may be objected that the sugar values obtained in this way would be too high both on account of ether reducing substances in the solution and because cold is not as efficacious as heat in stopping enzyme reaction. Cori (1925), however, in comparing the sugar content of various tissue extracts, before and after the removal of non-carbohydrate reducing substances with Lloyd's reagent, found that there was practically no "rest-reduction" in liver extracts, although in muscle extracts it was very marked. By

using lead acetate and alumina cream, on the other hand, Simpson and Macleod found considerable "rest reduction" in liver extracts. The values for free sugar which we obtained by this method are of that same order as those obtained by the majority of other workers.

Using the same extract, it was possible to determine the lactic acid content of each sample by treating the solution with copper sulphate and milk of lime, and following the Hirsch Kauffmann procedure (1924).

HYPERGLYCEMIA FOLLOWING SPLANCHNIC STIMULATION. Two experiments were carried out for the purpose of comparing the effects (on liver and blood) of splanchnic stimulation with those of asphyxia and adrenalin injection. As in previous experiments, two samples of liver and blood were

TABLE 3

Cat 20. Weight 3.2 kilos. Splanchnic stimulation and asphyxia

SAMPLE NUMBER	HOURS AFTER DECAPITATION	BLOOD SUGAR		WEIGHT OF LIVER	CARBOHYDRATE IN LIVER						TOTAL CARBOHYDRATE GLUCOSE
		Per cent	Grams		Glycogen		Free sugar		Lactic acid		
					per cent	*grams*	*per cent*	*gram*	*per cent*	*gram*	*grams*
1	$8\frac{3}{4}$	0.116	0.278	110	3.97	4.36	0.118	0.130	0.040	0.044	4.79
2	$9\frac{1}{4}$	0.120	0.288	$103\frac{1}{2}$	3.24	4.35	0.108	0.112	0.051	0.053	3.78
				d.							
		(Left splanchnic (nr. ganglion) stimulated for 5 minutes immediately preceding sample 3)									
3	$9\frac{3}{4}$	0.332	0.795	96	2.10	2.02	0.510	0.490	0.060	0.058	3.33
4	10	0.239	0.572	91	2.70	2.45	0.357	0.325	0.056	0.051	3.37
		(Asphyxia for 5 minutes and aeration for 2 minutes immediately preceding sample 5.)									
5	$10\frac{1}{2}$	0.348	0.834	71	2.28	1.62	0.809	0.574	0.100	0.071	3.06
6*	11	0.445	1.065	50	0.88	0.44	1.530	0.765	0.169	0.085	2.32

*Hemorrhage from liver occurring between the taking of samples 5 and 6, accounts for part of the glycogenolysis observed.

taken eight and eight and a half hours respectively, after decapitation, and hepatic free sugar as well as glycogen and blood sugar determined. Following this the splanchnic nerve on one side was stimulated at a point near the suprarenal ganglion, for 5 minutes in one case and for 10 minutes in the other. In the former case (table 3), the liver glycogen fell from 3.24 to 2.1 per cent in 15 minutes and then rose to 2.7 per cent in the next 15 minutes. The blood sugar rose from 0.12 to 0.33 per cent in the same time, and then fell to 0.24 per cent. The free carbohydrate showed the most marked change, rising from 0.11 to 0.51 per cent and subsequently falling to 0.36 per cent at similar 15 minute intervals. The lactic acid content remained unchanged at 0.05 per cent. In the other experiment similar but less pronounced effects were obtained.

About one hour after the direct splanchnic stimulation both preparations were asphyxiated for the customary 4 to 5 minutes. The effects were even more marked than those recorded above for splanchnic stimulation (table 3). In the first preparation the liver glycogen fell from 2.70 to 2.3 per cent in 15 minutes; and then to 0.9 per cent in the following half-hour, while the blood sugar rose from 0.24 to 0.35 per cent and ultimately to 0.45 per cent; the free carbohydrate rose from 0.36 to 0.81 per cent and then to 1.5 per cent. Even the lactic acid content rose from 0.06 to 0.10 per cent and eventually to 0.17 per cent during the same period of time. In the other preparation the results were similar, but less marked.

The effects of splanchnic stimulation observed by Macleod (1924) are not quite so pronounced as those recorded above for the first preparation, but are about the same as those we obtained on the second. Taking samples from the vena cava, which shows changes much sooner than arterial blood, Macleod obtained an average rise of 0.04 per cent in blood sugar in from five to ten minutes after stimulation. Using arterial blood, we obtained a rise of 0.21 per cent in 5 minutes in the glycogen rich cat, but a rise of only 0.04 per cent in 15 minutes in the glycogen poor cat. Thus the hyperglycemia of splanchnic stimulation resembles that of adrenalin injection, in appearing far more quickly and markedly in animals having a fair deposition of glycogen in the liver. When the necessary calculations are made it is again found that the glycogen broken down exceeds the glucose recoverable from the liver and blood stream. It is interesting to note that splanchnic stimulation did not in either case cause any change in the lactic acid content of the liver.

Cori, Cori and Pucher (1923) claim that even after 24 hours' starvation, the free sugar content of the liver is between 0.3 and 0.4 per cent. In only one of our experiments (cat 19) was so high a figure obtained after decapitation; the free sugar stood at 0.3 per cent, while the blood sugar was at 0.135 per cent. In the remaining cases the level of free sugar in the liver was the same as that in the blood, the two sugars being 0.11 per cent and 0.118 per cent in cat 20, and 0.097 and 0.098 per cent in cat 21. Subsequent changes in the free sugar resembled those in the blood sugar, but were usually more pronounced.

Observing carbohydrate metabolism in the liver, Cori (1923) found that after adrenalin injection there was a large rise in free sugar and a fall in glycogen, and that the peak of the blood sugar curve occurred later than that of the free sugar. We found that after splanchnic stimulation, and also after asphyxia, the same sequence of events occurred, and that the free sugar began to decline from its maximum before the blood sugar did. Evidently the former diffuses from the liver into the blood, causing hyperglycemia.

In contradistinction to splanchnic stimulation, asphyxia caused a de-

cided rise in the lactic acid content of the liver, amounting to 0.1 per cent in one hour. Cori (1925) claims that during asphyxia there is no change in the lactic acid content of the liver, but there is a distinct rise in that of the blood. Collazo (1925), however, believes that part of the hepatic function is to transform carbohydrates into lactic acid. In our experiments there was so little blood included with the liver tissue that it is doubtful whether the lactic acid content of the asphyxial blood could account for all the lactic acid found.

EFFECT ON CARBOHYDRATE BALANCE OF TAKING ACCOUNT OF CARBOHYDRATE LOST IN COMBUSTION. In one experiment the expired air was analysed and the loss of carbohydrate calculated by use of Lusk's well-known tables. It was thought that this might prove to be the missing

TABLE 4

Cat 29. Illustrating air analysis, and estimation of glucose lost in combustion

HOURS AFTER DECAPITATION	VOLUME EXCHANGE	OXYGEN USED		R.Q.	CALORIES		CALORIES CONSUMED AS CARBOHYDRATE		GLUCOSE CONSUMED
		Per cent by analysis	Litres per hour		Per litre O_2 (Lusk)	Per hour (actual)	Relative (Lusk)	Actual	
	litres per hour						*per cent*	*per cent*	
$1\frac{1}{2}$	60	1.93	1.16	0.81	4.813	5.58	35.4	1.975	0.49
3	60	2.04	1.22	0.80	4.813	5.86	32.0	1.875	0.47
4	60	1.68	1.01	0.77	4.764	4.81	21.8	1.049	0.26
$4\frac{1}{2}$	60	1.76	1.06	0.77	4.764	5.05	21.8	1.001	0.23
5	60	1.65	1.00	0.79	4.789	4.79	28.6	1.370	0.34
$5\frac{1}{2}$	60	1.57	0.94	0.79	4.789	4.50	28.6	1.287	0.32
6	60	1.54	0.92	0.77	4.764	4.38	21.8	0.956	0.24
$6\frac{3}{4}$	60	1.56	0.94	0.79	4.789	4.50	28.6	1.287	0.32

factor in our attempt to strike a balance in the carbohydrate metabolism of the decapitate cat.

A normal cat, starved for 24 hours, gave on three successive trials over a period of three hours each on different days R.Q.'s which showed almost no variation from the number 0.70. This same cat was used in the experiment. After decapitation its R.Q. varied from 0.81 at $1\frac{1}{2}$ hours, to 0.79 at $6\frac{3}{4}$ hours (table 4). The rate of disappearance of glucose through combustion was calculated from the known amount of oxygen absorbed and the R.Q. observed at different intervals. This is given in the last column of table 4. The blood sugar and liver glycogen and free sugar followed the same fluctuations that we had observed in the other preparations. The "total carbohydrate" in the last column of table 5 was obtained by adding the weight of sugar in the blood (the blood being estimated as $\frac{1}{13}$ the body

weight), the weight of glycogen and free sugar present in the remaining and functioning part of the liver, and the weight of the glucose lost in combustion up to that particular moment. If we are correct in assuming that muscle glycogen plays no part in these fluctuations in carbohydrate content of liver and blood, then one would expect the "total carbohydrate" to remain a constant quantity, since the preparation was not receiving carbohydrate from any outside source. But this was true only when the liver glycogen remained constant. As the glycogen rose to its usual maximum $6\frac{1}{2}$ hours after decapitation the "total carbohydrate" also rose, and as the liver glycogen fell after asphyxia, the "total carbohydrate" also fell, though it was compensated to a certain degree by the rise in blood sugar and still more in the second sample by the rise in free sugar.

TABLE 5

Cat 29. Weight 2.8 kilos. Carbohydrate present in liver and blood, and lactic acid combustion

HOURS AFTER DECAPITATION	BLOOD SUGAR		WEIGHT OF LIVER	CARBOHYDRATE IN LIVER				CARBOHYDRATE LOST IN COMBUSTION SINCE DECAPITATION	TOTAL CARBOHYDRATE GLUCOSE
				Glycogen		Free sugar			
	Per cent	Grams		Per cent	Grams	Per cent	Grams		
			grams						*grams*
$3\frac{1}{2}$	0.127	0.254	88.0	2.40	2.11	0.142	0.125	1.70	4.19
$4\frac{1}{4}$	0.140	0.280	83.0	2.80	2.32	0.189	0.157	1.90	4.66
$4\frac{3}{4}$	0.138	0.276	78.5	2.75	2.16	0.146	0.115	2.04	4.60
$5\frac{1}{4}$	0.128	0.256	73.5	2.80	2.06	0.098	0.072	2.21	4.60
$6\frac{1}{4}$	0.112	0.224	71.0	3.38	2.40	0.166	0.118	2.57	5.31
$7\frac{1}{4}$	0.094	0.188	66.0	3.10	2.05	0.170	0.112	2.81	5.17
(Asphyxia for $5\frac{3}{4}$ minutes followed by aeration for 2 minutes immediately preceding next sample)									
$7\frac{1}{2}$	0.321	0.642	62.0	(i) 1.65	1.02	0.128	0.080	2.89	4.63

The explanation of these results which seems to us most reasonable is that (always provided the muscles play no part in these changes) carbohydrate can exist in the organism in some form other than glycogen or glucose, which, therefore, escapes detection when the usual quantitative methods are employed for the determination of these carbohydrates. Any attempt on our part of further analysis of the phenomenon would be purely speculative, but a reversible reaction, whereby there could be rapid transformance of glucose or glycogen into the as yet unmeasurable and hypothetical form and *vice versa*, would account for the uncompensated decrease in liver glycogen after the point of maximum glycogen deposition had been reached, after injection of adrenalin, and after splanchnic stimulation.

SUMMARY

The endogenous hyperglycemias caused by anesthesia, asphyxia, adrenalin injection and splanchnic stimulation, are all associated with hepatic glycogenolysis. This breakdown of glycogen always exceeds the coincident increase of free sugar in the blood and liver, when the blood is taken as constituting $\frac{1}{13}$ of the body weight. There is, therefore, a certain percentage of carbohydrate lost during this process which cannot be accounted for.

Following decapitation there is a period of from three to eight hours during which glycogenesis exceeds glycogenolysis in the liver, the blood sugar meanwhile falling to a level slightly above normal. The blood sugar is obviously the source of some of this newly formed glycogen, but cannot account for all of it. This increase in glycogen is a consequence of decapitation since it is not produced by anesthesia alone.

Following the period of post-decapitation deposition of glycogen in the liver, hepatic glycogenolysis begins to exceed glycogenesis, and although no change in the level of the blood sugar occurs, the percentage of glycogen in the liver steadily falls. The one case in which the respiratory exchange was studied showed that it was impossible to account for the extra loss of carbohydrate through combustion, and since the blood sugar was below its renal threshold, there was no reason to believe that the glucose was being excreted in the urine.

Asphyxia and anesthesia are more effectual in causing hyperglycemia, than are adrenalin or splanchnic stimulation, at least for the conditions under which our experiments were carried out. Asphyxia is particularly pronounced in its effects and probably plays a major rôle in the hyperglycemia of anesthesia. Asphyxia during the period of diminishing liver glycogen (i.e., 8 to 12 hours after decapitation) frequently renders the liver almost glycogen-free; but during the period of glycogen deposition (i.e., from 1 to 8 hours after decapitation) the liver rapidly recovers from the slight glycogenolysis which takes place after asphyxia.

Asphyxia produces a marked and rapid rise in the concentration of free sugar in the liver, so that it may sometimes constitute 1½ per cent of the liver weight. There is simultaneously a small but definite increase in the lactic acid content, mounting to 0.1 per cent above the normal concentration.

Adrenalectomy abolishes the hyperglycemic effects of asphyxia for several hours, but the characteristic rise in blood sugar can be produced five hours after the operation. The initial absence of response may be due to post-operative shock. On the other hand the body may be able to adjust itself to the loss of the adrenal glands in so far as its reaction to asphyxia is concerned.

Adrenalin hyperglycemia depends to a considerable extent on the quan-

tity of glycogen in the liver. It is not so prolonged as the hyperglycemia of asphyxia.

The hyperglycemia following splanchnic stimulation is greatly influenced by the concentration of glycogen in the liver; it may be very marked, or scarcely noticeable. The free sugar of the liver rises more rapidly and to a higher level than that of the blood, and appears to diffuse into the blood stream. The lactic acid content of the liver is not affected by splanchnic stimulation.

BIBLIOGRAPHY

BANG, I. 1913. "Der Blutzucker." Wiesbaden.

COLLAZO, J. A. 1925. Compt. Rend. Soc. Biol., xlix, 407.

CORI, C. 1925. Journ. Biol. Chem., lxiii, 253.

CORI, C. F. AND G. T. CORI. 1925. Journ. Pharm. Exper. Therap., xxiv, 456.

CORI, C. F., G. T. CORI AND G. W. PUCHER. 1923. Ibid., xxi, 377.

DOWLER AND V. H. MOTTRAM. 1918. Journ. Physiol., lii, 166.

HIRSCH-KAUFFMAN, H. 1924. Pflüger's Arch., cciv, 295.

KELLAWAY, C. H. 1920. Journ. Physiol., liii, 211.

LANGFELDT, E. 1921. Journ. Biol. Chem., xlvi, 381.

MANN, F. C. 1925. Journ. Amer. Med. Assoc., lxxxv, 1472.

MACLEOD, J. J. R. 1913. Diabetes, its pathological physiology. E. Arnold, London.
1926. Carbohydrate metabolism and insulin. Longmans Green and Co., London.

MACLEOD, J. J. R. AND R. G. PEARCE. 1911. This Journal, xxvii, 341; xxviii, 403.

OLMSTED, J. M. D. 1926. This Journal, lxxv, 487.

PFLÜGER, E. 1905. Das Glycogen. Leipzig, Vogel.

RITZMANN, H. 1909. Arch. f. exper. Path. u. Pharm., lxi, 231.

SIMPSON, W. W. AND J. J. R. MACLEOD. (Unpublished.)

SOSKIN, S. 1927. This Journal, lxxxi, 382.

MICRO-INJECTION STUDIES OF CAPILLARY PERMEABILITY

III. The Effect of Lack of Oxygen on the Permeability of the Capillary Wall to Fluid and to the Plasma Proteins

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Received for publication October 18, 1927

There is ample evidence that tissue activity is accompanied by a change in the equilibrium between the circulating blood and the tissue fluids. It is an old observation, verified more recently by Barcroft and Kato (1916), that though lymph cannot be obtained from resting muscle except in small amounts, it flows freely during and after a series of contractions. According to Lazarus-Barlow (1894) muscular activity also increases the transudation produced after a short period of total anemia. While hemo-stasis alone has little effect upon transudation, when accompanied by muscular activity it causes a striking movement of fluid into the tissues giving rise to an obvious edema. Under these conditions there is evidence that the lymph becomes abnormally rich in protein. Lazarus-Barlow attributes the edema to alteration of the vessel walls by "tissue starvation."

The glomerular filtrate in the kidney with normal circulation is protein-free (Wearn and Richards, 1924). Starr (1926) has shown that stoppage or even reduction of blood flow through the kidney produces a transient albuminuria, which is not accompanied by any microscopically visible pathological change. It seems that the permeability of the glomerular capillaries is so increased by temporary asphyxia that when blood flow is reëstablished protein escapes from the plasma.

It has been shown (Landis, 1927b) that in the frog the rate of filtration of fluid through the capillary wall is directly proportional to the excess of capillary pressure over the osmotic pressure of the plasma proteins. Since accumulated metabolic products are rather generally believed to produce vasodilatation and hyperemia (Roy and Brown, 1879; Hooker, 1911; Harris, 1922; Hemingway and McDowall, 1926; Lewis, 1926) and since local active hyperemia is accompanied by a rise in capillary pressure (Landis, 1926, 1927a) an increased rate of filtration from the blood into the tissue spaces must result. This aids in explaining the increase in

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lymph flow which accompanies tissue activity. It does not explain, however, the presence of protein in the filtrates collected after periods of interrupted blood flow, since the normal capillary wall, even though dilated, seems to be impermeable to the colloidal constituents of the plasma (Landis, 1927b). Apparently, however, a sufficiently prolonged interruption of circulation, particularly if accompanied by tissue activity, renders the wall permeable to the plasma proteins.

Such a reduction in blood supply might increase the permeability of the capillary wall by reason of 1, lowered oxygen tension; 2, increased carbon dioxide tension, or 3, local increase in acidity due to the accumulation of metabolites. In the experiments described in this paper an attempt has been made to treat these factors separately in order to ascertain what effect each may have *a*, upon the movement of fluid into active tissues and *b*, upon the permeability of the capillary wall to the plasma proteins.

MATERIAL AND METHODS. As in previous experiments, the capillaries in the mesenteries of decerebrate frogs (Rana catesbiana and R. pipiens) were used throughout. The exposed mesentery was kept moist by a continuous flow of Ringer's solution. Observations were limited to single capillaries, and the rates of filtration or of absorption of fluid through the capillary wall were measured directly and computed in terms of cubic micra of fluid per square micron of capillary wall per second, according to the method described in a preceding paper (Landis, 1927b). Each measurement of fluid movement was followed by the direct determination of blood pressure in the observed capillary in a manner which has also been fully described (Landis, 1926).

1. The effect of oxygen-free Ringer's solution on fluid movement. The determination of the rate of fluid passage requires the stoppage of blood flow in single capillaries for a period of 60 to 100 seconds. The movement of the corpuscles at the open end of the obstructed vessel is used to indicate the direction and the rate of fluid movement through the wall. Immediately after the circulation through the capillary is stopped, fluid movement is rapid, then becomes slower, finally ceasing altogether as the colloid osmotic pressure of the blood imprisoned within the vessel comes into equilibrium with capillary pressure as a result of the concentration or dilution of the plasma proteins. Similarly the corpuscles at the open extremity of the capillary move rapidly at first, then more slowly and finally come to rest when the new colloid osmotic pressure balances the capillary pressure.

When the mesentery is bathed with well oxygenated Ringer's fluid the corpuscles retain this new position of equilibrium practically indefinitely. For instance, as shown by the solid lines in figure 1, at 8.7 cm. pressure, absorption caused the observed corpuscle to move outward quite rapidly during the first 20 seconds, then more slowly, ceasing finally at 60 seconds.

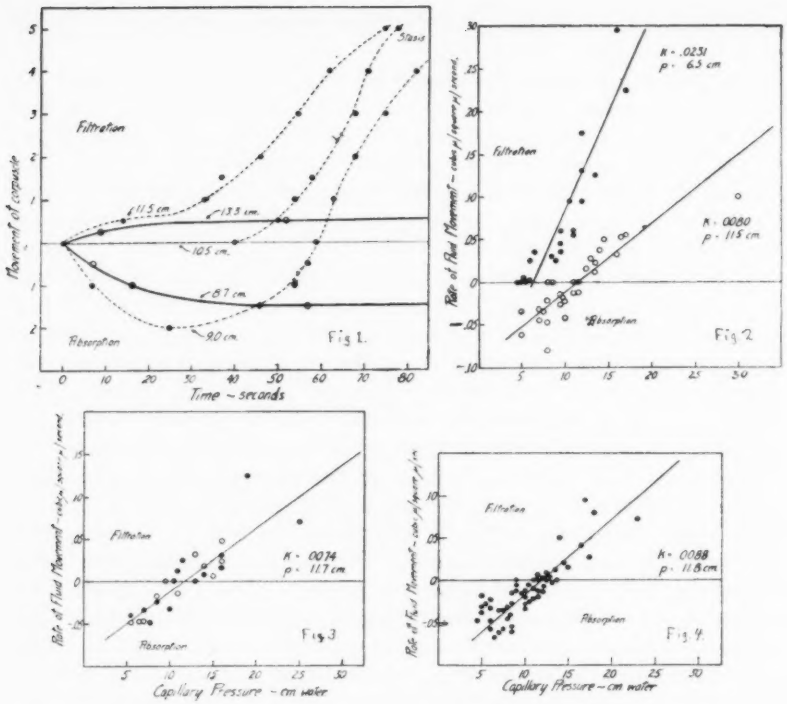

Fig. 1. Chart showing the effect of oxygen lack upon movement of corpuscles after stoppage of circulation. Abscissae represent movement of corpuscle at the free end of the capillary measured in ocular micrometer units, each equivalent to 33 micra; ordinates show time in seconds. Filtration was indicated by movement of corpuscle toward the closed end of the capillary, absorption by movement away from the closed end. Solid lines indicate fluid movement in mesentery covered with oxygenated Ringer's fluid; dotted lines in mesentery covered with boiled, oxygen-free Ringer's fluid.

Fig. 2. Chart showing rates of fluid movement observed one minute (dots) and 15 minutes (circles) after a 3-minute interruption of blood flow, while the mesentery was covered with boiled, oxygen-free Ringer's fluid. Abscissae indicate the rate of fluid movement in cubic micra per square micron of capillary wall per second; ordinates the capillary pressure in centimeters of water. k is the filtration constant computed from the slope of the straight line; p is the effective osmotic pressure of the plasma proteins.

Fig. 3. Chart showing rates of fluid movement observed one minute (dots) and 15 minutes (circles) after a 3 to 5 minute interruption of circulation while the mesentery was covered with boiled Ringer's fluid subsequently oxygenated. Symbols same as in figure 2.

Fig. 4. Chart showing the rates of fluid movement observed in capillaries of mesenteries covered with Ringer's fluid saturated with carbon dioxide. Abscissae represent fluid movement in cubic micra per square micron of capillary wall per second; ordinates the capillary pressure in centimeters of water. k is the filtration constant and p is the effective osmotic pressure of the plasma proteins.

This new position was maintained for a period of three minutes when observations were discontinued. At 13.3 cm. movement of the corpuscle was inward toward the closed extremity, indicating filtration, but very shortly equilibrium was established and the cell remained stationary for over three minutes. Apparently therefore in the presence of oxygenated Ringer's fluid the effective osmotic pressure of the plasma proteins is maintained in spite of the local stoppage of circulation.

A different situation exists when the mesentery is bathed with Ringer's fluid which has been boiled to remove dissolved gases, and subsequently cooled. The observed corpuscle at the free end frequently does not maintain its new position for more than 30 to 40 seconds, after circulation ceases. At the end of this period the cells move rapidly inward, indicating speedy filtration, which continues usually until nearly all the plasma has filtered through the wall. This filtration supervenes even at capillary pressures well below the osmotic pressure of the plasma proteins. Thus in the dotted lines of figure 1 are shown a series of determinations made in capillaries of a mesentery covered by Ringer's fluid which had been freed of its gases by boiling. At a pressure of 9 cm. the observed corpuscle moved outward toward the free extremity, indicating absorption of fluid. This absorption was at first rapid, then slower, but between 30 and 40 seconds after circulation had ceased it was quite suddenly transformed into filtration which continued until the corpuscles had been concentrated at the closed end of the capillary, though the pressure was well below the colloid osmotic pressure of the plasma proteins. Another instance is shown in which at 10.5 cm. neither filtration nor absorption occurred during the first 40 seconds, as would be expected since capillary pressure in this vessel approximately equalled the colloid osmotic pressure. At 50 seconds, however, rapid filtration began and ceased only when the corpuscles were packed in stasis due to the loss of most of the plasma. Again at 11.5 cm. slow filtration was changed into rapid filtration about 30 seconds after blood flow had been stopped.

This rapid filtration at low capillary pressures indicates a reduction in the effective colloid osmotic pressure of the plasma proteins. If the concentrated corpuscles are replaced by a new volume of blood and fluid movement again determined immediate and rapid filtration is always observed. The rate of filtration from this second volume of blood is practically the same as that observed during the latter part of the first period, even in capillaries which at first showed absorption of fluid. This indicates that the effect is not to be ascribed to change in the proteins of the imprisoned blood, but is probably due to a sudden increase in the permeability of the capillary wall, which then permits the free passage of plasma with its colloids. Since this increase in capillary permeability failed to appear when circulation was stopped in vessels bathed by oxy-

genated Ringer's fluid it seemed possible that the normal impermeability of the capillary wall to protein might be influenced by lack of oxygen.

The results obtained in this way were, however, not sufficiently uniform to indicate quantitatively the influence this factor might have upon fluid interchange, probably due to the diffusion of oxygen from adjacent capillaries, in which blood flow continued during the period of observation. The clearest examples of increase in permeability could only be obtained in capillaries far removed from other vessels. Therefore for a quantitative determination of the effect which lack of oxygen has upon the permeability of the capillary wall to fluid and to colloids it was necessary to stop circulation simultaneously throughout the whole mesentery.

2. The effect of oxygen lack on fluid movement and permeability to colloids. In studying the effects of lack of oxygen its transport to the capillaries by the blood stream was prevented by compressing the mesenteric artery and vein. The intestine was delivered through the usual lateral body wall incision and a loop of silk thread was passed about the vessels at the base of the mesentery. The two ends of the thread were then conducted through a glass tube of about 2 mm. diameter and 10 cm. length, and tied to a screw, by the rotation of which the thread could be tightened. The tube was fixed firmly with its end against the mesentery as it lay loosely on the glass stage. Tension applied to the thread pulled the vessels against the rounded end of the tube, thus quickly obstructing venous and arterial circulation without stretching the mesentery.

In figure 2 are shown the results obtained in a series of experiments in which circulation was interrupted for a period of three minutes, while the mesentery was bathed in Ringer's fluid which had been previously boiled and then cooled with suitable precautions against aeration. Figure 3 shows another series, conducted in an identical manner, with the single exception that the Ringer's fluid, previously boiled and cooled, was saturated with oxygen during the experiment.

Before measuring the rates of filtration it was necessary to permit the circulation to return in order that normal arterial blood might fill the capillary network. Within the first minute following this resumption of flow the rate of fluid movement was determined in one capillary. The rates of filtration observed in a series of vessels at this time are indicated in figures 2 and 3 as dots. It was essential to determine filtration as soon as possible after the termination of the period of anemia, since recovery followed rapidly upon the return of circulation, becoming almost complete often within 3 to 5 minutes. In order to determine the full degree of recovery possible a second series of determinations was made after normal flow had continued for 15 minutes. These values are shown as circles in figures 2 and 3. This determination was then followed by another period of anemia. In the absence of oxygen this sequence could be repeated from

3 to 4 times in the same mesentery apparently with almost complete recovery of the endothelium fifteen minutes after each interruption of circulation. With a greater number of repetitions, however, the results became erratic, indicating injury if the periods of asphyxia were too frequent.

When circulation is resumed immediately following the loosening of the ligature about the artery and vein, the velocity of blood flow is very much more rapid than before, producing a definite reactive hyperemia. The capillaries, already moderately dilated by the exposure of the mesentery, very rarely show any increase in diameter as a result of the anemia, so that the effects observed are not to be attributed to dilatation of the capillaries. Apparently the increased blood flow is the result of dilatation proximal to the capillary net work, possibly resembling the changes described under certain conditions in the kidney by Richards and Schmidt (1924).

When the mesentery is bathed with oxygen-free Ringer's fluid (fig. 2) the observed rates of filtration after an asphyxia of 3 minutes' duration fall obviously into two groups, depending on whether the determinations are made during the first or during the fifteenth minute after the end of the period of asphyxia. When plotted against capillary pressure the observations in each group are distributed about a straight line. It has been shown (Landis, 1927b) that the rate of fluid movement through the normal capillary wall is directly proportional to the difference between the capillary pressure and the osmotic pressure of the plasma proteins, or that:

$$\text{Rate of fluid movement} = k\,(P - p)$$

where P represents capillary pressure in centimeters water, and p the effective osmotic pressure of the plasma proteins, measured against the capillary wall as a filter. The term k, which may be called the "filtration constant," can be computed from the slope of the straight line drawn through the observed points according to the method of least squares. In a series of 70 observations on normal capillaries k had a mean value of 0.0056. This figure represents the rate of fluid movement in cubic micra per square micron of capillary wall per second, for a difference of 1 cm. water between capillary pressure and the colloid osmotic pressure.

During the first minute after the period of anemia the slope of the line is greater than normal (fig. 2) indicating an abnormally rapid increase in the rate of filtration with rise in capillary pressure. The computed k is 0.0231 compared to the normal of 0.0056, indicating a four-fold increase in the rate at which fluid moves through the wall with unit difference of pressure. After the fifteen minute recovery period, however, k has decreased to 0.0080, which is only slightly greater than the normal figure.

The point where the straight line intersects the line of zero filtration represents the effective osmotic pressure of the plasma proteins. Immedi-

ately after the period of asphyxia the effective osmotic pressure is lowered from the normal of 11.5 cm. water to 6.5 cm. Fifteen minutes later it has returned again to the normal level of 11.5 cm. as a result of the resumption of circulation. This lowering of the effective osmotic pressure of the plasma colloids might conceivably arise in several ways such as 1, change in the blood itself, particularly of the plasma proteins; 2, the accumulation in the tissue spaces of metabolic products, and 3, increase in capillary permeability of a degree sufficient to permit in part the passage of the plasma proteins, thus lowering their effective osmotic pressure. The evidence is all in favor of the last explanation.

The first possibility does not apply since before each determination the capillary network was filled with normal arterial blood. Moreover, in the first series of experiments described above it was found that in the absence of oxygen rapid filtration was observed not only from the blood first imprisoned within the capillary but also occurred immediately and rapidly if a fresh volume of blood was introduced into the same vessel, showing that the effect was localized in the capillary wall, or outside it.

However, if the lowered effective osmotic pressure were due to the accumulation of products of metabolism immediately outside the capillary wall, it would be indicated solely by a shifting of the line toward the left. In the absence of a modification of the capillary wall the slope of the line would be entirely unchanged and the filtration constant would necessarily be the same as that of the normal wall. This, however, is not the case since the filtration constant is over four times as great, indicating definitely a modification of the capillary wall in the direction of greater permeability to fluid. Apparently this change is sufficient to permit the plasma proteins to escape. In addition, lack of oxygen is very effective in increasing the rate of filtration of such highly colloidal dyes as brilliant vital red and trypan blue. Finally, the mesentery is composed of cells whose metabolism is of a very low order, and it seems unlikely that the extravascular accumulation of metabolites could amount to the equivalent of 4 to 5 per cent of protein within a three minute period.

A number of factors, however, might conceivably be involved in a permeability change following stoppage of circulation. That the effect is really due to lack of oxygen alone became apparent when a control series of experiments was carried out under identical conditions except that the Ringer's fluid, having been boiled, then cooled, was thoroughly oxygenated during the course of the experiments. In figure 3 the dots show rates of filtration immediately after the period of interrupted blood flow, which in some instances was increased to five minutes; the circles show rates after a fifteen minute recovery period. Both series of observations, with a single exception, are distributed about the same straight line. The slope yields a filtration constant of 0.0074, which is only slightly

greater than the normal of 0.0056. The difference is probably due to the manipulation necessary in preparing the mesentery. The effective osmotic pressure is 11.7 cm. water and therefore approximately normal. The fact that the entire effect of interruption of circulation could be abolished by the use of oxygenated Ringer's indicates that the change in permeability was primarily due to lack of oxygen.

The increase in capillary permeability has also been obtained by stopping circulation in the presence of Ringer's fluid through which nitrogen was being passed. In general a longer period of anemia was required, 10 minutes with nitrogen-washed Ringer's fluid being the equivalent of 3 minutes with boiled Ringer's fluid. Possibly the difference was due to traces of oxygen in the nitrogen, which was not specially purified.

The same effects, though to a less marked degree, were also observed after shorter periods of circulatory stoppage. Even 1.5 to 2 minutes of anemia were sufficient to increase capillary permeability slightly but with these shorter periods the change in permeability disappeared so quickly that it was very difficult to determine it quantitatively. On the other hand an anemia of 5 to 10 minutes' duration produced in many instances an irreversible stasis, with a loss of plasma equal to that observed after injury with 10 per cent alcohol, or 1:10,000 mercuric chloride. In a very few capillaries this stasis disappeared following the application of oxygenated Ringer's fluid, though usually recovery was not observed. Even after stasis had resolved the wall remained abnormally permeable to both fluid and protein.

It seems from these experiments, therefore, that when circulation is stopped for a period of three minutes, in the absence of oxygen, there is a marked increase in the permeability of the capillary wall. This results not only in the more rapid passage of fluid, but also in the loss of protein, with a consequent reduction of the effective colloid osmotic pressure of the plasma. The change seems to be reversible within certain limits, so that fifteen minutes later both the rate of fluid movement and the effective colloid osmotic pressure have returned to normal. This indicates not only that the wall is again impermeable to colloids but also that whatever protein passed through the wall during and after the period of asphyxia was no longer present in the tissue spaces immediately surrounding the capillary.

2. The effect of increased carbon dioxide tension on capillary permeability. Though it seems clear that the change in permeability observed after stoppage of circulation must be due primarily to oxygen lack it is nevertheless necessary also to determine the possible effect of other factors which may affect the capillary wall simultaneously. In the intact animal both tissue activity and the interruption of circulation result in a local increase of carbon dioxide tension. In order to test the effect that this

factor might have upon the capillary wall the exposed mesentery was bathed with Ringer's fluid saturated to varying degrees with purified carbon dioxide.

The Ringer's solution contained sodium bicarbonate sufficient to produce a pH of 8.2 which in solutions fully saturated with carbon dioxide was reduced to between 5.1 and 5.2, and when half saturated to approximately 5.4. The mesentery was exposed in the usual manner and covered by a continuous and rapid stream of Ringer's fluid containing carbon dioxide. The first determinations of fluid movement were made only after the mesentery had been bathed in the solution for 10 to 15 minutes.

Various concentrations of carbon dioxide, up to half saturation, produced no measurable change either in the rate of fluid movement, or in the effective colloid osmotic pressure. The mesenteric circulation was to some extent modified by dilatation of the capillaries, and a more rapid flow of blood through the capillary network. The relation between rate of fluid movement and capillary pressure, however, remained entirely normal.

Fully saturated Ringer's solution produced a very slight increase in the rate of filtration of fluid at any given capillary pressure. Thus the slope of the line in figure 4 is slightly greater than normal, the filtration constant being 0.0088 as compared to the normal of 0.0056, so that for any given difference between capillary pressure and colloid osmotic pressure there is only a 40 per cent increase in the rate of fluid movement. The effective osmotic pressure as indicated by the line in figure 4 is 11.8 cm. water. It is higher by 0.3 cm. than the figures obtained for several series of normal capillaries, but it is doubtful whether the difference is sufficiently great to be significant.

It seems evident that even saturation with carbon dioxide, while it increases the rate of filtration very slightly, does not do so to a degree comparable with lack of oxygen. Moreover increase in carbon dioxide tension cannot explain the movement of protein through the walls of capillaries in which blood flow has for some time been interrupted. It appears that the changes in carbon dioxide tension which lie within the physiological range do not influence the movement of fluid by any direct effect upon capillary permeability, but only in an indirect manner through the rise in capillary pressure which accompanies hyperemia.

4. The effect of increased acidity on capillary permeability. Increase in hydrogen ion concentration due to the accumulation of acid metabolites is another factor which might possibly modify the capillary wall during tissue activity, or during periods of anemia. Since the physiological range of hydrogen ion concentration is quite restricted this factor might conceivably have marked influence upon the endothelium.

The mesentery was bathed with unbuffered Ringer's fluid to avoid the

addition of ions other than the ones previously used. The solution was brought to various pH levels by addition of hydrochloric acid and then oxygenated. An abundant flow of fluid was provided since it passed over a square centimeter of the mesentery at the rate of a liter in 30 minutes. This produced a constant and rapid renewal of the solution and buffering seemed unnecessary. The pH of the fluid was tested both before and after it had passed over the tissue.

Table 1 gives the results of a series of determinations at various pH levels, showing the filtration constant and the effective osmotic pressure obtained from plotted figures similar to those given above. There appears to be little change over the range from 8.0 to 4.5. The effective osmotic pressure remains at the normal level throughout. There is a very slight rise in the rate of fluid movement indicated by the increase in k from the normal of 0.0056 at 8.0 to 0.0074 at 5.0. Thus at a given difference between capillary pressure and colloid osmotic pressure the rate of fluid movement will be increased over normal only by a third with a change of 3 units in pH.

TABLE 1

pH OF RINGER'S FLUID	NUMBER OF OBSERVATIONS	FILTRATION CONSTANT, k	EFFECTIVE OSMOTIC PRESSURE, p
			cm. water
8.0	70	0.0056	11.5
6.0	58	0.0065	11.7
5.0	28	0.0074	11.4
4.0	28	0.0152	11.6
3.5	22	0.0207	7.8
3.0		Rapid stasis	

The first clear indication of modified permeability appeared at a pH of 4.0, with an increase in the filtration constant to 0.0152, indicating that fluid movement took place at a rate almost three times as rapid as that observed at pH 8.0. The effective osmotic pressure of the proteins, however, remained at the normal level of 11.6 cm. showing that the increase in permeability was not sufficient to permit the passage of proteins. At pH 3.5, however, there is not only a further increase in the rate of fluid movement, but also a partial reduction in the effective colloid osmotic pressure, indicating that the wall had become permeable to protein. This was obvious on microscopic observation by the ease with which vessels passed into stasis when circulation was stopped for the determination of fluid movement. At 3.0 measurements became quite impossible due to marked injury which caused the erythrocytes to adhere to the capillary walls.

The interpretation of these results is made less easy by reason of the fact that in order to reach the capillary wall it is necessary for the acid to penetrate a layer of mesothelial cells. It seems unlikely, however, that increased acidity can be a factor of any great importance in the modification of capillary permeability observed during and following interruption of circulation. Significant changes were observed only at pH levels far outside the physiological range and were then clearly due to injury.

Discussion. That local asphyxia might increase the permeability of the capillary wall has been suggested frequently to explain certain effects of impeded circulation, particularly the free passage of fluid, and often of protein, into the tissue spaces. By direct analysis of the glomerular filtrate Wearn and Richards (1924) found that the glomerular capillaries of the frog are impermeable to protein. The normal capillary wall of the frog's mesentery apparently remains impermeable to protein even during dilatation, since the effective osmotic pressure of the plasma proteins remains the same irrespective of diameter (Landis, 1927b). Under these conditions hyperemia alone cannot add to the protein content of the filtrate. It has been observed in fact with moderate muscular activity, that the amount of protein in the lymph is reduced (Hamburger, 1893; Pugliese, 1898), which becomes explainable only on the basis of a more rapid filtration of fluid containing little or no protein.

The fluid filtered through the capillary wall may, however, contain abnormally large amounts of protein. According to Japelli and d'Errico (1908) the lymph flowing from the thoracic duct post mortem is at first watery, but after a few minutes its viscosity begins to increase, rising finally to that of blood serum. This was ascribed to the increase of dissolved substances particularly of proteins Bainbridge (1906) found that physical factors otherwise explained the flow of lymph post mortem and suggested that lack of oxygen increased the permeability of the visceral capillaries. The experiments of Starr (1926) already cited show clearly the close relation between circulatory obstruction and the increased permeability of the glomerular capillaries.

The direct measurement of fluid movement through the walls of single capillaries, with the direct determination of capillary pressure, indicates that lack of oxygen alone is capable of producing an increase of permeability which is sufficient in degree to permit the passage of protein. The accumulation of carbon dioxide and of acid products, certainly within the physiological range, have almost no effect upon fluid movement. The passage of protein, identified by others by testing the filtrate, became apparent in these experiments in a quantitative reduction of the effective colloid osmotic pressure.

This effect of oxygen lack is of particular interest since it seems probable that the edema of cardiac decompensation cannot be explained purely in

terms of a rise in capillary pressure. Though venous pressure is uniformly elevated during the accumulation of edema fluid (Eyster and Middleton, 1924; Boas and Dooneief, 1924; Meldolesi, 1926), there is no general agreement concerning capillary pressure which may be elevated (Meldolesi, 1926) or may be lower than normal (Boas and Dooneief, 1924). Moreover Bolton (1903, 1910, 1915) found that partial obstruction of the circulation through the inferior vena cava produced an edema which appeared even with a normal venous and a subnormal arterial pressure. He suggested therefore that the collection of fluid was probably independent of capillary pressure and was due to damage of the capillary walls, though the rapidity with which recovery was observed following the return of circulation caused him to hesitate somewhat in adopting this view.

It has been found also that the edema fluids resulting from myocardial insufficiency contain a relatively high percentage of protein, in many instances only slightly less than the concentrations found in true inflammatory fluids (Beckmann, 1921; Haas, 1921; Loeb, Atcheley and Palmer, 1922). It is interesting to note in this respect that in these quantitative experiments upon single capillaries the reversible increase in permeability after a period of oxygen lack is not quite equal to the irreversible change produced by death of the endothelial cells. Thus k after 3 minutes' oxygen lack is 0.0207, whereas after death from alcohol or 1:10,000 mercuric chloride k is 0.0260 (Landis, 1927 b). The permeability of the wall to protein following oxygen lack reduced the effective colloid osmotic pressure to 6.5 cm. water, but injury with alcohol or mercuric chloride diminished it to 4.0 cm. The experiments which have been described above indicate the probable importance of oxygen lack as a factor in producing the changes in fluid movement observed during cardiac decompensation. In regions with sluggish blood flow oxygen lack might well produce permeability changes, particularly on the venous side of the capillary network, or in capillaries in which the intermittency of flow was unduly prolonged.

The rapid recovery observed by Bolton (1915) following the resumption of normal circulation can be explained on the basis of the remarkable degree of reversibility observed in the asphyxial changes in the mesenteric capillaries. Within limits it appears that recovery to normal is almost complete, certainly it is entirely complete so far as permeability to protein is concerned. Apparently therefore the plasma proteins can pass through the temporarily asphyxiated capillary wall, though the same wall under conditions of sufficient blood flow and ample oxygenation will still retain its normal impermeability to proteins. To what extent this modification of capillary permeability may influence the movement of fluid, and possibly of protein, into actively functioning tissues under physiological conditions cannot at present even be surmised.

It was mentioned that fifteen minutes after the asphyxial period the

colloid osmotic pressure returned to normal indicating not only that the wall was impermeable to colloids but that the fluid immediately outside the capillary was free of protein, though previously the wall permitted its passage. Both the lowered effective osmotic pressure during asphyxia, and the higher capillary pressure of reactive hyperemia, would tend to increase the rate of filtration of fluid, which would contain less and less protein as the capillary wall recovered its normal impermeability. This protein-free filtrate would thus wash the protein-containing fluid out of the tissue spaces immediately surrounding the capillary into the lymph vessels. Drinker (1927) and Churchill, Nakazawa, and Drinker (1927) suggest that the relatively high protein content of the fluid taken from the subcutaneous lymph spaces of the frog is evidence of the high permeability of the skin capillaries, which they suggest apparently "leak protein-containing fluid at all diameters." It seems possible, however, from the recovery described above that the tissue fluid immediately outside the capillary walls can differ widely in composition from the lymph even of the same area. In addition the original filtrate might be modified considerably during its passage through the tissue spaces, the smaller lymph capillaries and lymph glands. Until the absence of these changes is proved it is doubtful whether comparison of lymph and plasma can in itself yield definite information concerning the permeability of the capillary wall. Since the capillaries in two situations as different as the mesentery and the kidney appear to be impermeable to protein, it seems possible that this may prove to be a general property of the endothelial wall. It is obviously unjustifiable, however, to generalize too far on this basis, and further experiments on capillaries in other tissues must be performed.

Finally in preparations of the mammalian mesentery (Florey, 1926) and the frog's mesentery (Landis, 1927a) isolated capillaries were found in which the plasma was for a time almost completely filtered off, leaving merely a solid mass of corpuscles within the vessel. In certain instances where this condition of "stasis" appears without obvious cause there is spontaneous resolution. If permeability is measured during the period of stasis it is found to be much increased, while after resolution it usually approaches the normal. These changes in permeability, as mentioned by Florey (1926), occur without measurable change in diameter. It seems possible that local asphyxia may explain the temporary increase in permeability and the loss of plasma. As the surrounding circulation improves the diffusion of oxygen from adjacent vessels may restore the normal grade of impermeability to the wall, which then becomes capable of supporting circulation without excessive loss of protein or of fluid. The fact that in a few instances stasis produced by lengthy periods of oxygen lack resolved when the mesentery was covered with oxygenated Ringer's fluid suggests

the validity of this explanation of the spontaneous resolution of stasis in apparently normal vessels.

The observations which have been described emphasize further the individual character of the responses which are to be expected in the capillary network, and make more evident the advantage of observing singly the units of fluid interchange. It appears that oxygen lack, in addition to its indirect effects through the tissue metabolites upon capillary diameter and rate of flow, also directly increases the permeability of the capillary wall, permitting not only the more rapid filtration of fluid but even the passage of the plasma proteins. The asphyxiated capillary wall resembles the normal vessel in that fluid movement is directly proportional to the difference between capillary pressure and the effective osmotic pressure of the plasma proteins, though in the asphyxiated vessel the latter pressure is lowered by the passage of protein through the capillary wall which is, however, still merely a passive filter, though more permeable than normal.

SUMMARY

The effects upon capillary permeability of oxygen lack, of high tensions of carbon dioxide, and of increased hydrogen ion concentration were studied in single capillaries of the frog mesentery, by the direct measurement of fluid movement in cubic micra per square micron of capillary wall per second, with measurement of capillary pressure.

Immediately after a three minute period of oxygen lack fluid filtered through the capillary wall at approximately four times the normal rate. The increased permeability of the wall permitted also the passage of protein, thus reducing the effective osmotic pressure of the plasma proteins to almost one-half their normal value.

The movement of fluid through the asphyxiated capillary wall was directly proportional to the difference between capillary pressure and the effective osmotic pressure of the plasma proteins, indicating that the wall still acted merely as a passive filter, though more permeable than normal.

With the return of circulation the capillary wall rapidly recovered its impermeability to protein and the rate of fluid movement was also reduced practically to normal.

Exposure of the mesentery to Ringer's fluid half saturated with carbon dioxide produced no change in fluid movement. Complete saturation increased the rate of fluid movement very slightly, but the wall remained normally impermeable to protein.

Increase in hydrogen ion concentration produced almost no change in fluid movement within physiological limits, though at pH 4.0 the characteristic effects of injury appeared.

Certain conditions in which the capillary wall becomes permeable to protein are discussed in view of these observations.

BIBLIOGRAPHY

BAINBRIDGE. 1906. Journ. Physiol., xxxiv, 275.
BARCROFT AND KATO. 1916. Phil. Trans. Roy. Soc. London, B. ccvii, 149.
BECKMANN, K. 1921. Deutsch. Arch. f. klin. Med., 1921, cxxxv, 39.
BOAS AND DOONEIEF. 1924. Arch. Int. Med., xxxiii, 407.
BOLTON, C. 1903. Journ. Path. and Bact., ix, 67.
1910. Journ. Path. and Bact., xiv, 49.
1915. Journ. Path. and Bact., xx, 290.
CHURCHILL, NAKAZAWA AND DRINKER. 1927. Journ. Physiol., lxiii, 304.
DRINKER, C. K. 1927. Journ. Physiol., lxiii, 249.
EYSTER AND MIDDLETON. 1924. Arch. Int. Med., xxxiv, 228.
FLOREY, H. 1926. Proc. Roy. Soc., B. c, 269.
HAAS, C. 1921. Verhand. d. deutsch. Gesellsch. f. inn. Med., xxxiii, 410.
HAMBURGER. 1893. Zeitschr. f. Biol., xxx, 143.
HARRIS, D. T. 1922. Proc. Roy. Soc., B. xciii, 384.
HEMINGWAY AND MCDOWALL. 1926. Journ. Physiol., lxii, 166.
HOOKER, D. R. 1911. This Journal, xxviii, 361.
JAPELLI AND D'ERRICO. 1908. Zeitschr. f. Biol., l, 1.
LANDIS, E. M. 1926. This Journal, lxxv, 548.
1927a. This Journal, lxxxi, 124.
1927b. This Journal, lxxxii, 217.
LAZARUS-BARLOW, W. S. 1894. Phil. Trans. Roy. Soc. London, B. clxxxv, 779.
LEWIS, T. 1926. Heart, xiii, 1.
LOEB, ATCHELEY AND PALMER. 1922. Journ. Gen. Physiol., iv, 591.
MELDOLESI, G. 1926. Cuore e circolazione, i, 20; ii, 45.
PUGLIESE, A. 1898. Pflüger's Arch., lxxii, 603.
RICHARDS AND SCHMIDT. 1924. This Journal, lxxi, 178.
ROY AND BROWN. 1879. Journ. Physiol., ii, 323.
STARR, I. 1926. Journ. Exper. Med., xliii, 31.
WEARN AND RICHARDS. 1924. This Journal, lxxi, 209.

THE RELATION BETWEEN WORK PERFORMED AND HEAT LIBERATED BY THE ISOLATED GASTROCNEMIUS, SEMITENDINOSUS AND TIBIALIS ANTICUS MUSCLES OF THE FROG

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Received for publication October 19, 1927

It has long been known, from the investigations of Fick (1878), Heidenhain (1864) and others, that muscles liberate varying amounts of heat with varying loads even though the duration of the stimulus remains constant. This fact was rather lost sight of for many years while attention was directed to improvements in myothermic technique and particularly to the study of the isometric contraction by Hill and Hartree. Renewed investigations of the heat produced in isotonic contractions in the sartorius muscle (Fenn, 1923, 1924) showed that when work was done by the muscle, there was liberated heat in excess of the heat produced in an isometric contraction, and that this excess heat was related in a very complicated way to the load lifted, the work done and the time after stimulation when shortening took place. When, on the other hand, the muscle was stretched during contraction, less heat was liberated than when the muscle was allowed to shorten. This result has been confirmed by Azuma (1924) and in some of its aspects by Hartree (1925) and Wyman (1926).

Under the conditions of these experiments it appeared that the excess heat was nearly equal to the work performed by the muscle and the suggestion was made 1, that the excess heat was liberated, so to speak, for the purpose of doing work, and 2, that it thereby became difficult to consider a muscle as doing work at the expense of elastic potential energy previously stored up within it at the time of stimulation. It is perhaps not surprising that this hypothesis has not been universally accepted. The tendency is rather (Hill, 1925; Wyman, 1926) to retain the very convenient elastic body theory and to reconcile it with the heat measurements by assuming that the total energy transformed into mechanical potential energy is independent of the load but that in an isometric contraction some of this mechanical potential energy reverts to chemical potential energy without appearing as heat. This hypothesis is quite as good and quite as complicated as the "work" hypothesis, but there does not seem to be any conclusive evidence as yet in favor of either of them.

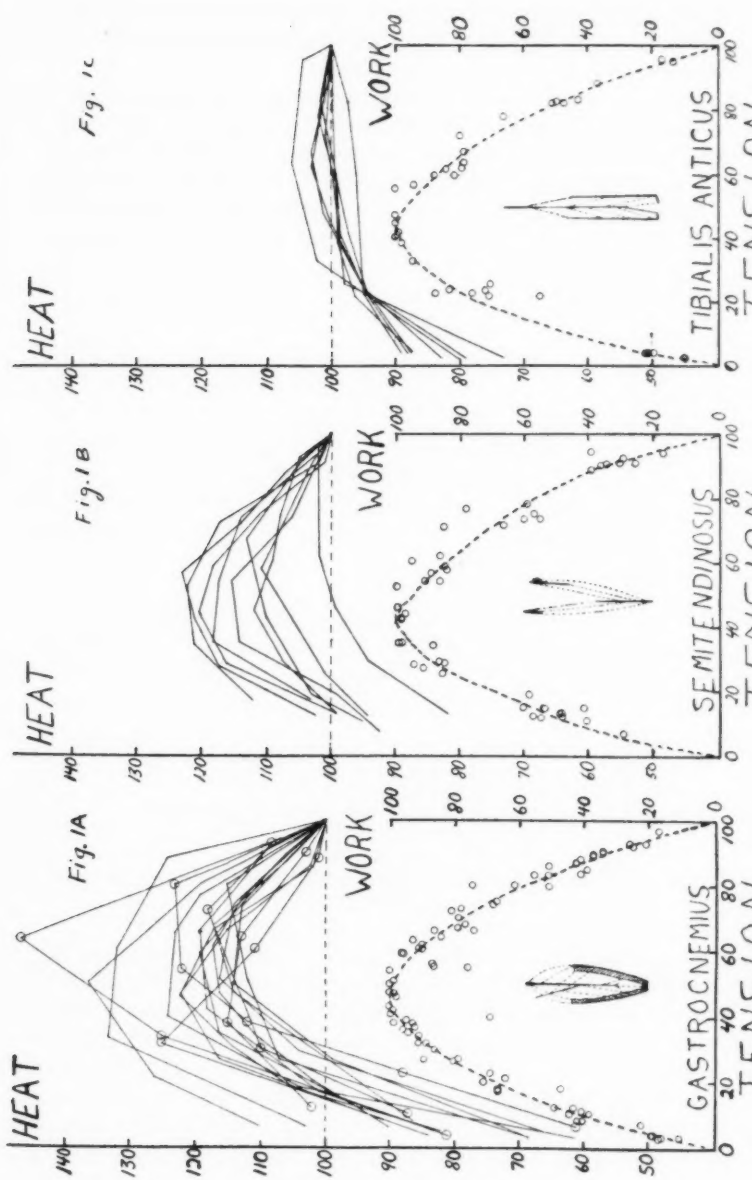
The theoretical importance of such an increased energy transformation during work is, therefore, far reaching but the question arises as to how generally true it is. As a contribution to this aspect of the subject, the heat production of the gastrocnemius, semitendinosus and tibialis anticus muscles of the frog has been investigated by methods similar to those described by Fenn (1923). Older work on the heat production of muscles other than the parallel-fibred sartorius were carried out by Heidenhain (1864), Fick (1878, 1884, 1892), Greife (1895), Bürker (1905, 1919), Frank (1904) and others with quite conflicting results. In some cases more than one muscle was used in the preparation; in other experiments the series with increasing loads were not carried completely to the isometric load, and in others, the initial length was not controlled by any after-loading device.

EXPERIMENTAL. The thermopile used had 64 silver-constantan junctions and a resistance of 34.3 ohms. The galvanometer was of the D'Arsonval type, critical damping resistance of 40 ohms, and a sensitivity of 5.05 $\times 10^{-9}$ amperes at 1 meter distance with the thermopile in circuit. The deflection was read at a distance of 4.25 meters from the mirror, giving a sensitivity of 1.19 $\times 10^{-9}$ amperes. The muscles were kept at a constant temperature for the few seconds required for measuring the initial heat by an air-stirred water bath kept at room temperature—approximately 21°C. The duration of stimulus was kept constant by a timing device and the muscle was usually stimulated for 0.2 second by a just maximal alternating current of 60 cycles per second.

Observations were made of the galvanometer deflection and of the corresponding amounts of shortening (smoked drum record) of the muscles when stimulated to lift a series of weights increasing to the isometric. These results were averaged with those obtained in a similar series taken in the reverse order. Because of the irregular shapes of these muscles, no attempt was made to calibrate them, and because they differed so widely in size and strength, the relative shapes of their heat curves are best compared by plotting them as percentages of the isometric heat. Similarly, the load is expressed as percentage of the just isometric load, and work as percentage of the maximum work done by the muscle under optimum load.

Figures 1A, 1B and 1C show all the curves obtained in this way. No significance is to be attached to the closeness of the experimental points on the work curve to the average work curve, since three points on this curve are arbitrarily fixed by the percentage method of plotting. The diagrams of the structure of the muscles were copied from Beritoff (1925). Figure 2 shows the average heat curve of each muscle compared with the sartorius curve measured by Fenn.

DISCUSSION. There is a tendency in all these curves toward a maximum heat production at the point of maximum work, yet there is no parallelism



Figs. 1A, 1B, 1C. Solid lines represent heat curves; broken lines, work curves. Ordinates, heat in percentage of isometric heat, work in percentage of maximum work under optimum load. Diagrams of muscle fibres copied from Retzoff. Circles around individual points on heat curve in figure 1A represent values obtained with indirect stimulation.

except in the sartorius curve. Each of the muscles studied has a characteristic curve, possibly depending upon the arrangement of the fibers in the muscle.

Since in the sartorius muscle, shortening involves an extra liberation of energy, it seemed possible that the relatively high isometric heat of the other three muscles (compared with isotonic heat) might be due to a shortening of the individual fibres even though the muscles were rigidly fixed at both ends. In an attempt to test this possibility, silhouettes of each muscle were taken in the resting state and in the state of isometric

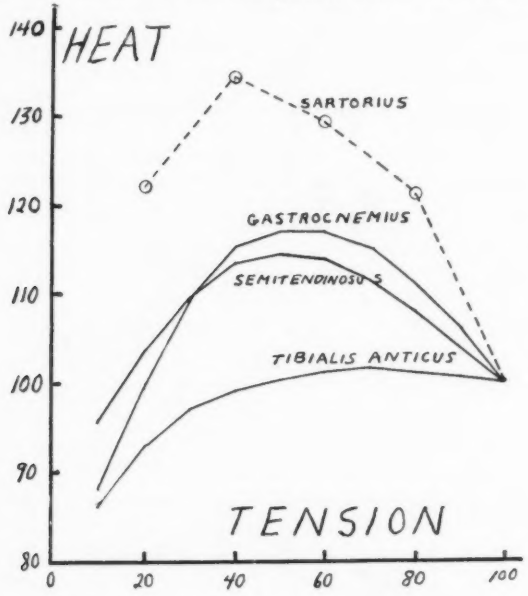


Fig. 2. Average heat curves of each muscle, compared with the sartorius curve (broken line).

contraction. Since internal work cannot be measured quantitatively, even from photographs, it hardly seems worth while to reproduce them. There is some internal shortening in every muscle, including the sartorius, which shows some fibres shortening at the expense of others, even when the muscle is stretched to 110 per cent of its natural resting length. The gastrocnemius shows the most movement of all, chiefly in the nature of a telescoping of the outer sheath over the central tendon. The tibialis anticus shows a shifting of the central tendon to one side, probably causing a considerable expenditure of internal work since the tension and amount of shortening are considerable.

Unless it is assumed that the individual fibres in different muscles react differently to changes in mechanical conditions, one must look, for an explanation of these variations, to the anatomical arrangement of the fibres in the four muscles. At present it seems impossible to find any sufficiently quantitative basis for such an explanation, although it can hardly be doubted that the explanation is fundamentally a structural one. Whether such an explanation is consistent with the hypothesis that the excess heat is related to work must remain for future experiments to decide.

SUMMARY

1. The relation between work performed and total energy liberated by the isolated gastrocnemius, semitendinosus and tibialis anticus muscles of the frog, has been investigated.

2. There is a characteristic heat curve for each of the muscles studied.

3. It is suggested that the difference in these heat curves may be due to the anatomical arrangement of the muscle fibres in question.

I am indebted to Doctor Fenn for his help and many suggestions during this investigation.

BIBLIOGRAPHY

AZUMA, R. 1924. Proc. Roy. Soc. B., xcvi, 338.
BERITOFF, J. 1925. Pflüger's Arch., ccix, 763.
BÜRKER, K. 1905. Ibid., cix, 217.
1919. Ibid., clxxiv, 282.
FENN, W. O. 1923. Journ. Physiol., lviii, 175.
1924. Ibid., lviii, 373.
FICK, A. 1878. Ges. Schrift., ii, 363.
1884. Verhandl. der Phys.-med. Gesell. zu Würzeburg, N. F. xix, 61.
1892. Pflüger's Arch., li, 541.
FRANK, O. 1904. Ergebn. d. Physiol., iii, 2.
GREIFE, H. 1895. Pflüger's Arch., lxii, 111.
HEIDENHAIN. 1864. Mechan. Leistung, Wärmentwickelung u. s. w. Leipzig (available only in quotations by other authors).
HARTREE, W. 1925. Journ. Physiol., lx, 269.
HILL, A. V. 1925. Ibid., lx, 237.
WYMAN, J. 1926. Ibid., lxi, 337.

THE INFLUENCE OF THE PROPRIOCEPTIVE NERVES OF THE HIND LIMBS UPON THE POSTURE OF THE FORE LIMBS IN DECEREBRATE CATS

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Received for publication October 18, 1927

Orderly locomotion among the quadripeds is secured through accurate coördination of rhythmic movements of the fore and hind limbs. Many observations have been made in the past upon the coördination of the musculature in the two hind limbs and in the two fore limbs, but few facts are on record concerning the influence of the hind limbs on the fore limbs, and vice versa. The following observations have to do with the influence of the proprioceptive nerves arising in the quadriceps extensor muscle upon the resting posture of the fore limbs. The results, which have been observed consistently in twelve preparations, may best be illustrated by giving the protocol of one experiment.

Wednesday, May 18, 1927. A large female cat was decerebrated by the trephine method at 9:35. Prior to the decerebration the following nerves on the left side were severed: sciatic, pudendal, all branches of the hamstring nerve except that passing to the semitendinosus, external cutaneous, internal saphenous, obturators, and nerve to sartorius. The rotators of the hip were all immobilized by tendon resection, and the tensor fasciae femoris was freely resected. On the right side the hamstring, sciatic, anterior crural and external cutaneous nerves were severed, but the adductor muscles retained their nerve supply (obturator nerve). The adductors on the right, and the left semitendinosus and quadriceps were thus the only muscles of the hind limbs which were not immobilized.

This preliminary dissection was carried out under deep ether-chloroform narcosis, and the time elapsing from the beginning of the anaesthesia to the completion of the dissection and the decerebration was just twenty minutes. Every effort has been made during the past few weeks to minimize the duration of the anaesthesia, for it has seemed to us that the animals survive for a much longer period when the anaesthesia is brief. After decerebration the preparation, with its head held high, had its cranial vault packed with severed pieces of its own temporalis muscle on top of which dry cotton was placed. The preparation was then carefully wrapped with heavy cotton-batting inside of which warm bottles were placed, and permitted to remain undisturbed with its head still held high until 1 p.m. At that time there was no evidence of intracranial haemorrhage and the animal was apparently in excellent condition.

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Observations on reflexes. Before isolating the tendons of the left quadriceps and semitendinosus the effect of elicitation of the lengthening reaction in the left quadriceps upon the posture of the fore limb was carefully studied. We observed that sudden forceful flexion of the knee-joint against the resistance offered by quadriceps (*i.e.*, the lengthening reaction; (11), 3,)) produced:

1, extension of the fore limb on the same side.

2, flexion of the fore limb on the opposite side. These postures continued to be maintained so long as the hind limb was held forcefully flexed, but if the hind limb were slightly relaxed the fore limbs returned to their resting posture even before the hind limb was released from its position of forced flexion, but this was not usual. The response in the fore limbs to the lengthening reaction induced in one of the hind limbs, occurred both when the position of the head was normal and when rotated away from the limb which was being manipulated, but the reaction was much decreased in intensity when the chin was rotated toward the stretched quadriceps. These responses were constant and striking even though the preparation as a whole did not at this time exhibit much "spontaneous" alteration in the distribution of its tonus when the head was rotated (7), (10).

The tendons were isolated at 1:50 (quadriceps and semitendinosus). Between 2 and 3 o'clock the following observations were made:

1. Moderate traction on the patellar tendon produced: *a*, extension of the fore limb on the same side; *b*, flexion of the opposite limb,—observations which might have been predicted from the behavior of the intact limb when forcefully flexed.

2. Weak faradization (Harvard coil coreless at 8.5 cm. fed by 2-volt accumulator with 10 ohms resistance in primary circuit; make shocks cut out by Sherrington separation key) of the ipsilateral sciatic nerve trunk produced the same response in the fore limbs, and the effect was less with the head rotated toward the side stimulated both with faradization and with patellar traction (as before). Faradization of the cut twigs of the hamstring produced less of an effect than faradization of the sciatic or either of its two main constituents (n. peroneal or popliteal).

3. Traction of the semitendinosus, the knee flexor, on the other hand, had no demonstrable effect upon the fore limbs even though 700–800 gm. tension was applied to the tendon. This we look upon as of importance since it excludes the possibility that the result observed with traction on the extensor tendon was due to secondary stimulation of the skin, bone or cut areas around the incisions. Also, when the flexor was stretched during a stretch of the extensor, there appeared to be no diminution in the extensor's effect upon the fore limbs.

4. Pressure *confined to the patellar tendon alone* (without traction upon the muscle as a whole) served to produce the effect on the fore limbs that has just been described. The pressure of one's finger-nail on the under surface of the patella elicited the reaction. Pressing the tendon with a pair of forceps was equally effective. It was also observed that the reaction could be obtained by pressing the body of the muscle between one's thumb and finger, without in any way increasing the tension upon the tendon. All forms of stimulus were less effective when the head was rotated toward the muscle on which the manipulation was being carried out.

5. The effect on the fore limbs was associated with a simultaneous Phillipson's reflex in the crossed hind limb, as inferred from the strong contraction of the adductors which occurred on that side. Had the anterior crural nerve not been cut the Phillipson's reflex would undoubtedly have affected the right quadriceps muscle.

At 3:30 the preparation was set up and the muscles attached to the double isometric recording lever and responses obtained to weak faradic stimulation of the ipsilateral sciatic nerve with and without the head rotated. The reversal of ex-

tensor response with rotation of the head (10) noted in many previous preparations was not marked in this animal, though it was present to a slight degree. However, as the preparation was in excellent condition at 6 o'clock, we took it down from the myograph, closed up the incisions, covered it well and permitted it to stay until morning.

At 9 a.m. the next morning (May 19th) the preparation was still in good condition, breathing quietly. On examination of the reflexes it was found that they were all more or less uniformly increased. Thus, in the fore limbs vigorous tendon reflexes were obtainable from slight taps on either the triceps or biceps tendon. Jerks were also obtained from the extensors and flexors of the digits in the fore limbs. From the isolated tendon of the left semitendinosus a well-marked jerk reflex was obtained from a slight tap. This continued to be elicitable for about fifteen minutes, but with disturbance of the preparation incident to exposing the nerve trunks this tendon response vanished, at least we were not able to obtain it when the muscle was attached to the myograph. The influence of traction upon the patellar tendon on the fore limbs was still present, and the threshold for the elicitation of the reaction was very low. Pressure confined to the patellar tendon was still effective in producing the reaction, and traction upon the flexor tendon did not alter the character of the response. The influence of the neck reflexes was more marked this morning than it had been the night before, and records were accordingly taken to illustrate the effect of rotation of the head upon the flexors and extensors respectively.

The preparation stopped breathing at 11:30 and autopsy showed absence of hemorrhage into the cerebral cavity, but there was a small clot underneath the tentorium pressing upon the right cerebellar hemisphere. The level of transection had passed through the middle of the anterior colliculus but the brain stem for 3 mm. posterior was oedematous and obviously not in a state of functional activity.

The experiment of May 18th is referred to at length because this preparation lived longer than other preparations which we have studied, and, since its physical condition (as inferred from pulse, respiration and blood pressure) was unusually good throughout the period of observation, we look upon the results obtained in this case as being significant. As the observations recorded in this protocol have been repeatedly confirmed in subsequent as well as in previous experiments, it is permissible to make certain deductions.

There is in the first place the outstanding fact that stimulation of certain afferent nerves arising in the quadriceps muscle produces prompt extension of the ipsilateral fore limb and flexion of the opposite fore limb. This observation throws important light upon the reflex regulation of progressive movements in quadripeds, and it also raises a question as to the type of proprioceptive influence responsible for producing this reflex.

The importance of these reactions in progressive movements is obvious, for when the lengthening reaction is elicited in one quadriceps (*e.g.*, through application of vigorous extension) the limb itself becomes flexed, *i.e.*, lifted from the ground. At the same time the ipsilateral fore limb becomes extended,—"pillar-like" as Magnus (8) expressed it,—and is thus prepared to support the weight of the fore part of the body. Owing, however, to

Phillipson's reflex (9) the *opposite hind limb* becomes simultaneously extended thus giving support to the hind quarters of the body. Finally, the opposite fore limb, which at the same time becomes flexed, is made ready for another step. We see at first hand in these reactions of the four extremities a fundamental principle of quadriped stepping movements: viz., that at any given instant the weight of the fore and the hind quarters of the body tends to be supported chiefly by upper and lower extremities on opposite sides of the body. We do not wish to infer that this particular reaction dominates the locomotor mechanism, nor does it account for the rhythmicity of stepping (see 12, 1, 3). Undoubtedly there are other reciprocal reflexes (which have not yet been studied in detail), *e.g.*, of the fore limbs upon the hind limbs. Forceful flexion of the fore limbs, for example, is capable of inducing changes of posture in the hind limbs, and it is undoubtedly through the interplay of these reciprocal mechanisms that locomotor movements are regulated.

It is evident that the reactions described in this communication are brought about by excitation of a somewhat special functional group of proprioceptive end-organs. The end-organs which subserve the knee-jerk are not responsible for the reaction for, in our experience, the knee-jerk is not associated with any change in the position of the upper extremities. Similarly, a mild stretch reflex is not associated with the reaction, but once the extending force is sufficiently intense to induce a lengthening reaction, which, as one of us has pointed out elsewhere (3, ch. xvii), is due simply to a reflex inhibition, then and only then is the fore-limb reaction to be observed. Be it noted that the fore limb reaction is always associated with a simultaneous Phillipson's reflex (crossed extensor response of the hind limb (9)). Reasons have been given elsewhere (3) for the belief that Phillipson's reflex results from the stimulation of proprioceptive inhibitors, and among the proprioceptive inhibitors are those nerve endings known as the "nociceptive," the psychical adjunct of whose stimulation is pain. We have seen, however, that stimulation of the central end of the hamstring nerve and of the sciatic nerve also induces the reaction. It would seem evident, therefore, that nociceptive stimulation, whether of proprioceptive origin or of cutaneous origin, gives rise to the same fundamental reaction: a thorn in an animal's foot will produce it as well as a strain on its tendon.

Careful examination of available literature reveals that there are surprisingly few references to the reciprocal effects of the hind extremities upon the upper extremities, and vice versa. We find that Spiegel and Worms (13), in their studies upon the reflexes of decerebrate animals, have noted that stimulation of the central end of the sciatic leads to reflex rotation of the fore limbs on the opposite side. They state that this is also associated with slight flexion, but no mention is made of the

application of these observations to the mechanism of progressive movement (cf. Girndt, 5).

Important will be the determination of the spinal pathways involved in this proprioceptive reflex. Since the nociceptive fibres appear to be among those responsible for the reaction, even though they are proprioceptive in origin, it is likely that they pass to the fore limb through the intersegmental association bundles in close proximity to the spinothalamic tracts. We believed at first that the reflex was destroyed by removal of the cerebellum, but later preparations did not confirm this suspicion and lately in another investigation one of us, in association with Harmon (6), noted that the fore limb effect above described was to be seen in high spinal preparations, but it was poorly maintained and much less ample than the corresponding response observed in decerebrate animals. Extension of the ipsilateral fore limb on faradization of the central end of the sciatic nerve was obvious in the spinal preparation. There was also rather feeble contralateral flexion. In several preparations six to eight hours after the spinal transection there was also feeble contralateral extension of the hind limb. We believe, therefore, that the pathways of this reflex are chiefly intraspinal, though the reactions may be facilitated by higher reflex centers, just as with the stretch reflex (see Denny-Brown and Liddell, 2).

SUMMARY

1. Forceful flexion of the knee-joint of a decerebrate cat against the resistance offered by the quadriceps muscle causes: *a*, extension of the fore limb on the same side; *b*, flexion of the fore limb on the opposite side; and *c*, extension of the hind limb on the opposite side (Phillipson's reflex).

2. Vigorous traction on the isolated patellar tendon, or pressure confined to the tendon itself without extension of the muscle as a whole, produces the same result.

3. Weak faradization of the central end of the sciatic nerve or of any of its constituents also produces the reaction.

4. Vigorous traction upon the semitendinosus or upon any of the knee flexors is without effect upon the fore limb and does not influence the opposite hind limb.

5. Active tendon reflexes are to be observed in the knee flexors after recovery from shock.

6. From these observations it is inferred that the reaction described under 1 above throws important light upon the mechanism of progression in quadripeds.

7. The paths for the reflex described in 1 and 3 above are chiefly intraspinal.

BIBLIOGRAPHY

(1) Brown, T. G. and C. S. Sherrington. 1912. Journ. Physiol., xliv, 125.
(2) Denny-Brown, D. and E. G. T. Liddell. 1927. Journ. Physiol., lxiii, 144.
(3) Fulton, J. F. 1926. Muscular contraction and the reflex control of movement. Baltimore: Williams & Wilkins, 644 pp.
(4) Fulton, J. F. and J. Pi-Suñer. 1928. This Journal, lxxxiii, 554.
(5) Girndt, O. 1926. Pflüger's Arch., ccxiii, 427.
(6) Harmon, P. M. and J. F. Fulton. 1927 This Journal, lxxxi, 487.
(7) Magnus, R. 1924. Körperstellung. Berlin: Julius Springer, xiii, 740 pp.
(8) Magnus, R. 1926 (Cameron Prize Lectures, 1926) Lancet, ii, ccxi, 531; 585.
(9) Philippson, M. 1905. Travaux du Lab. de Physiol., Institute Solvay, Bruxelles, Publiés par P. Heger, vii, pt. ii, 1–208.
(10) Pi-Suñer, J. and J. F. Fulton. 1928. This Journal (in press).
(11) Sherrington, C. S. 1909. Quart. Journ. Exper. Physiol., ii, 109.
(12) Sherrington, C. S. 1910. Brain, xxxiii, 1.
(13) Spiegel, E. and R. Worms. 1927. Pflüger's Arch., ccxvi, 432.

A NOTE CONCERNING THE PROBABLE FUNCTION OF VARIOUS AFFERENT END-ORGANS IN SKELETAL MUSCLE

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Received for publication October 18, 1927

The following observations, made in the course of another investigation (16), appear to us to throw light upon the function of certain of the afferent end-organs in skeletal muscle. The first observations relate to the nature of the sensory endings responsible for mediating the knee-jerk and the stretch reflex.

An intact extensor muscle of a decerebrate animal exhibits, when led off to a galvanometer, a series of small irregular action currents, as was first pointed out by Dusser de Barenne (7) and Buytendijk (4). If the tendon of such a muscle is detached from its anatomical insertion and allowed to shorten freely, no action currents are visible (9), (10), (11) provided the head is not rotated (16). When the muscle of such a preparation is stretched even slightly, irregular action currents reappear. If now a series of knee-jerks is elicited from the stretched muscle, large action currents occur at the beginning of each jerk. *During the jerk, however, the irregular action-current vibrations, present before the jerk began, are absent* (fig. 1); but after the muscle has commenced to relax the irregular electrical responses once more begin (fig. 1). The time of reappearance of the electrical responses in the course of relaxation varies with the extent of shortening permitted during the contraction. Thus in one experiment, where 0.05 mm. shortening occurred during the jerk, they began again at approximately "half-relaxation," but, where only 0.02 mm. shortening took place, action currents are not apparent until about "three-quarters relaxation." The first action current to appear is usually relatively large as contrasted with those which follow, and precedes by a constant interval a "hump" on the tension record. This hump has been observed previously (24), (3), being sometimes referred to as the "myotatic appendage" (3), (10), and is a constant feature of the knee-jerk (or ankle-jerk) of a decerebrate preparation. After the hump the irregular string oscillations so characteristic of antigravity muscles of decerebrate preparations again

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ensue (fig. 1). We wish to offer the following interpretation of these observations.

That electrical responses should be absent during the knee-jerk, reappearing only after relaxation has begun, yields information concerning the mode of stimulation of the receptors for the knee-jerk and for the stretch reflex.

In discussing the problem of the knee-jerk and stretch reflex receptors, a distinction must first be made concerning the mechanical conditions prevailing 1, in a muscle in which tension is increased passively by an extending force applied to the tendon, and 2, in a muscle whose tension is increased through active contraction. If tension alone were the adequate

Fig. 1. A. Series of knee-jerks of a decerebrate cat with chin rotated toward the recording quadriceps (see ref. 16). From above downward are the shadows 1, myograph (upper part retouched); 2, galvanometer string (mag. × 450; 8 mm. per m.v. tension); 3, line of zero mechanical tension; moment of application of single break shock to ipsilateral sciatic nerve indicated by arrow. (When the head is turned such a stimulus causes reflex contraction of the knee extensor rather than inhibition.) Vertical lines = time 0.02 second. Note the relative quiescence of string during knee-jerk, especially in those occurring during the contraction induced by the break shock; observe also the hump at the end of relaxation and its associated action current.

stimulus for the afferent end-organs responsible for these "myotatic" reactions, then either mode of tension-increase would be effective in eliciting such a "myotatic" reflex. Were this true one would conceive of the muscle receptors subserving this reflex as arranged in series (fig. 2) with tension-supporting elements (*e.g.*, compare the end-organs in the tendon). But our observations indicate that the active contraction of a previously stretched muscle apparently removes the stimulus from the "stretch receptors," since during the course of such a contraction the action currents, which on good evidence (10), (8) one associates with reflexes set up by these receptors, are absent. Therefore, it is likely that the sensory

organs in question are arranged, not in series, but *in parallel*[2] with the executant units of the muscle (fig. 3). Reference to figures 2 and 3 will make clear the two possibilities of arrangement of sensory endings in muscle. If in figure 3 the middle unit, *s*, which is sensory, is stimulated by a stretching force and in this way there is set up a reflex contraction in

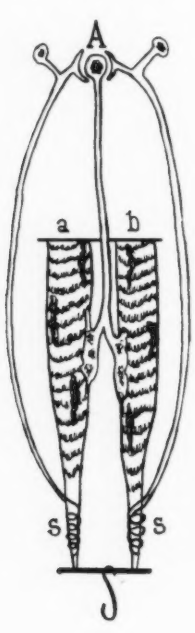

Fig. 2

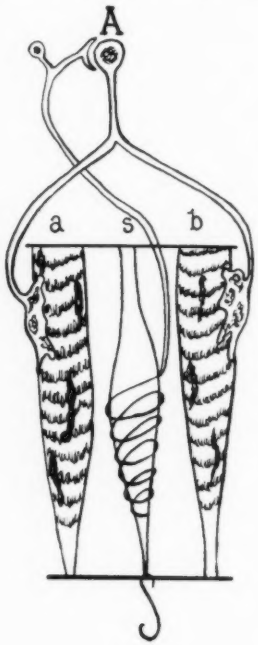

Fig. 3

Fig. 2. A diagram showing the mechanical conditions obtaining when muscle end-organs are disposed "in series" with the tension supporting elements. Under these circumstances tension, whether active or passive, will affect the end-organs indiscriminately. *a* and *b*, skeletal muscle fibres; *A* anterior horn cell.

Fig. 3. A diagram to show the mechanical conditions obtaining when a muscle end-organ is arranged "in parallel" with the tension supporting elements. *a* and *b*, skeletal muscle fibres; *s*, afferent end-organ associated with a fibre running parallel with *a* and *b* (*e.g.*, an intrafusal fibre); *A*, anterior horn cell.

muscle units *a* and *b*, it is evident that while *a* and *b* are active they will resist the stretching force applied at the tendon, and so operate to prevent further stimulation of *s*. When, however, *a* and *b* begin to relax, part of the burden of resisting the stretching force once more falls upon *s*, which

[2] We wish to acknowledge our indebtedness to Dr. Hallowell Davis for suggesting the use of the terms "in series" and "in parallel" in this exposition.

again becomes stimulated and there occurs a repetition of the previous process. Now the knee-jerk and the stretch reflex appear to be explicable upon the basis of just such a mechanism as that postulated in figure 3, and it is, moreover, difficult to account for them on the basis of any other mechanism.

When the muscle is placed under moderate initial tension, certain of its "stretch-afferent organs" are from time to time stimulated, and in this way give rise reflexly to the irregular action currents of the so-called "tonic" muscle (7). When a knee-jerk occurs, a large number of stretch afferents are set off in unison with the result that active tension is developed simultaneously in a large number of muscle fibres. While this internal tension lasts the external stretching force does not affect the afferent end-organs, and it is for this reason that we believe them to lie in parallel with the contractile elements which produce and maintain active tension. During relaxation, however, the myograph lever, by virtue of the potential energy stored in it during the jerk, vigorously extends the muscle, and in this way affects the end-organs lying in parallel with the previously active fibres (fig. 3). The result is a further reflex contraction (the "myotatic appendage" or "hump") with its associated action current. By application of an ipsilateral break shock of suitable intensity the myotatic appendage may be inhibited (3), (10) without inhibiting the jerk itself. The action current associated with the hump is then also absent and the myograph sinks, during relaxation, to a level lower than that from which the knee-jerk started, showing that a "tonic" substratum of contraction existed upon which the knee-jerk was superimposed. This latter "substratum of contraction" is due in large measure to a stretch reflex (15), (10), being the continuous response to a constant stretch. The knee-jerk, according to this interpretation, is a brief manifestation (15), (10) of the same underlying response.

1. *The muscle spindles.* When we come to correlate these observations with known morphological facts concerning the afferent endings in muscle we find certain highly suggestive relations. In the first place, muscle end-organs are divisible into two groups. *a.* Those which are disposed *in series* with the contractile active units; these are presumably tension recorders which provide for the central nervous system sensory data concerning tension alterations (active and passive). Of this group the most important are the Golgi tendon organs, which are found most abundantly in regions of the tendon and tendon aponeuroses immediately adjoining the flesh of the muscle. *b.* The second group are those disposed *in parallel* with the muscle fibres and correspond closely with the type of end-organ postulated in figure 3. These consist chiefly of the muscle spindles. We have accordingly a strong *a priori* ground for believing that the muscle spindles are in fact the end-organs which mediate such myotatic responses

as the knee-jerk and stretch reflex. It is therefore permissible to examine other evidence bearing upon this point.

In the first place the knee-jerk occurs in the absence of the tendon, for this structure can be entirely cut away without impairment of the response (24). One can therefore largely exclude as being essential for the jerk such end-organs as are peculiar to the tendon. This indicates that the end-organs within the fleshy parts of the muscle are responsible for the jerk. Of these undoubtedly the most common is the muscle spindle, but there are other afferent end-organs described by Regaud and Favre (20) which may conceivably be essential for the reflex. Sherrington (21) has pointed out that the properties of the spindle seem admirably suited structurally for response to mechanical, and especially to stretch, stimuli. The spindle is long and contains within it the successive folds of the sensory nerve ending. One would anticipate that a force tending to lengthen such a structure might stimulate its receptive endings. We have recently found, however, that gentle pressure upon the muscle, such as may be exerted between one's thumb and finger, will serve to elicit, if sudden, a response not unlike the knee-jerk,[3] and, if gradual, a response similar to the stretch reflex. It would be easy to imagine that such a deforming pressure stimulates the muscle spindle, but one must not conclude that lateral deformation is in itself the stimulus, for pressure applied transversely causes the muscle to become elongated beneath the fingers just as a soft rubber eraser becomes elongated when similarly pressed.

The schema postulated in figure 3 suggests that the reflex effect of stimulation of the afferent organ, *s*, may be localized in character, and this in point of fact is a prominent characteristic of the stretch reflex, for the response is strictly confined to the portion of the muscle which is stretched (15).

Several further points may also be mentioned as favoring the view that the spindles are responsible for the knee-jerk. The brief latency of the knee-jerk is a matter of common knowledge (see especially Jolly, 14). It is so brief, in fact, that a conduction rate of 80 to 100 meters per second is required in order to permit of its being reflex. The afferent nerve fibres supplying the muscle are of varying diameters (21), (22), and from the investigations of Gasser and Erlanger (12) we know that the fibres of larger diameter have higher conduction rates than those which are smaller. Consequently it is a point of some importance that the muscle spindles are in connection with the afferent nerve fibres of greatest diameter (21). Moreover, the afferent endings supplied by the fibres of 3 or 4μ diameter cannot be responsible for the jerk, since their conduction rate, as estimated by the cathode ray oscillograph (12), definitely precludes such a possibility.

[3] Dr. D. Denny-Brown of Oxford has written one of us that he too has lately made the same observation.

It may be mentioned in passing also that Adrian and Zotterman (1) have observed that single muscle spindles of a frog do in fact respond to passive stretch, but in such an experiment one has no way of determining what the reflex effect of such stimulation would be.

A word may be said concerning the distribution of the spindles in the various muscles of the body. They have been described in almost every muscle of the four appendages but are particularly abundant in the more distal muscles (*e.g.*, of the digits) (20), (22). They are absent from the extrinsic ocular muscles (22) and from the muscles of expression (20), (22), but are abundantly present in the muscles of mastication. It is perhaps significant that those muscles from which spindles are absent are little if any affected by gravity and would not therefore be expected to respond to stretch reflexes. This does not mean that one would anticipate absence of spindles from flexor muscles, for flexor muscles under certain circumstances give well-marked jerk responses (2), (17), and stretching them passively gives rise to other well-marked reflexes (6), (10). On the other hand, muscle spindles surround a contractile structure, the intrafusal fibre, and this suggests that they may signal active contraction, but it is only fair to say that we have no evidence that the intrafusal fibres ever do contract, and furthermore the region of the intrafusal fibres which is surrounded by the muscle spindle is without obvious cross striation, suggesting that in this region at least they cannot contract. But it must be admitted frankly that the function of the intrafusal fibres remains still a mystery, especially so since, unlike all other fibres in a muscle, its motor supply is probably from the sympathetic system (10) and since the intrafusal fibre itself does not degenerate after section of the motor nerve (21).

It may be urged that the absence of action currents during the knee-jerk is due to self-inhibition arising within the contracting muscle. That active contraction is capable of producing autogenous inhibitory effects cannot be doubted (6), (10, ch. xvii), and indeed this may be in part responsible for the absence of action currents, but it is difficult, on the basis alone of autogenous inhibition, to account for the regular reappearance of action currents during the relaxation of the knee-jerk and the variation in the time of their appearance with the shortening permitted during the contraction. It may be mentioned also that the action currents similarly disappear when the muscle is connected with the isometric lever well below its tendon, so that the effect cannot be attributed to the tendon organs present above the point of attachment.

Byrnes (5) has observed inflammation of muscle spindles in paralysis agitans, and yet in this disease knee-jerks are usually still present. This might appear to preclude the possibility of the spindles being responsible for the knee-jerk, but the degeneration which Byrnes describes is not complete and such a pathological process could not well be expected to exclude all such afferent end-organs.

Finally we are fully conscious of a further criticism which may be urged, —namely, that active contraction may stimulate the muscle spindles through lateral compression. One can only say that there is no good evidence that stimulation does occur in this way but there is nothing as yet to exclude the possibility.

2. *The tendon organs.* What function can be assigned to the end-organs which are in series with the active constituents of the muscle? We have suggested that they are tension recorders which supply to the central nervous system data concerning active and passive alterations in muscle tension. One of the significant features subjectively concerning tendons is that they give rise to pain when pressure is exerted upon them. One can readily demonstrate this by pressing upon one's tendon of Achilles. However, other qualities of sensation are undoubtedly mediated by these endings, for the eye muscles, which are without muscle spindles, possess large numbers of the Golgi tendon organs. The accurate adjustments of the eye are carried out unconsciously and only during spasm or fatigue is one conscious of the pain which may arise from the extraocular muscles. It may be urged that so long as the tension exerted upon the tendon bundles is moderate the sensual data originating from this tension does not reach consciousness, but when severe it does so. In view, however, of the definite segregation of pain endings in other parts of the body, it would seem more likely that specific pain endings likewise exist in muscle. Indeed examination of the relative diameters of afferent fibres in muscle is highly suggestive, for, if one observe the nerve to a muscle which has been deprived of its anterior root supply three months previously, the remaining sensory fibres in the nerve trunk vary widely in diameter (21). It is known that those of large diameter subserve the muscle spindles. No function has been assigned to those of intermediate (10μ) and small (3 to 4μ) diameter. Since in the fifth nerve the less differentiated endings, known to conduct "pain" impulses, are associated with fibres of small diameter, one would naturally presuppose that the small fibres arising in muscle subserved a similar function.

Direct evidence that end-organs associated with the tendon and with the aponeurotic attachments give rise to inhibition of the muscle itself was provided by Sherrington (23, p. 150). Thus he noted in the rigid vastocrureus muscle of a decerebrate preparation, that "running the end of a blunt seeker along the lines of attachment of the muscle at the sides of the lower part of the shaft of the femur causes smart partial elongations of the muscle. These reactions are obviously reflex inhibitions" and, as he points out, appear to be definitely referable to excitation of inhibitory endings belonging to the muscle itself. An observation which also establishes the existence of autogenous inhibitors arising in muscle is that faradic stimulation (*e.g.*, a single break shock) of the central cut end of one of the

branches of the nerves to quadriceps similarly gives rise to reflex inhibition of the remaining parts of the muscle (22), (10). We have observed also that crushing of the freshly isolated tendon gives rise to inhibition of rigidity, which points to the existence of inhibitory endings within the tendon. One of us has recently noted in a series of clinical cases exhibiting patellar and ankle clonus that one of the most effective ways of inhibiting either form of clonus is by pinching the tendo Achillis. It will be recalled that clinicians use insensitivity to strong pinch of the tendo Achillis as evidence of posterior root degeneration.

SUMMARY

When an extensor muscle of a decerebrate preparation is placed under slight initial passive stretch, irregular action currents are detectable in the galvanometer record. If a series of knee-jerks is elicited from a muscle under these circumstances, though large action currents precede the action current of each jerk, the irregular vibrations of the tonic muscle are absent during the jerk but reappear in the course of its relaxation. This is interpreted as indicating that during a knee-jerk the adequate stimulus is removed from the stretch afferent end-organs. A discussion is given of the nature of an end-organ which is responsive to passive stretch but unresponsive to tension set up by active contraction. Arguments are set forth to show that such an end-organ must exist *in parallel* with the tension-yielding elements of the muscle, for if, as with the tendon organs, it were disposed *in series* with these elements, no distinction could be made between the tension of a passive stretch and that of active contraction. Histologically the most important group of end-organs existing *in parallel* with the striated muscle fibres are the muscle spindles. In view of this and of other evidence discussed in the text, it is concluded that the muscle spindles are the receptors for the knee-jerk and the stretch reflex. The tendon organs are evidently the tension recorders, and it is also possible that they give rise to impulses which lead to reflex inhibition of the muscle itself. Whether or not they mediate pain, is left an open question.

BIBLIOGRAPHY

(1) Adrian, E. D. and Y. Zotterman. 1926. Journ. Physiol., lxi, 151.
(2) Asayama, C. 1915. Quart. Journ. Exper. Physiol., ix, 265.
(3) Ballif, L., J. F. Fulton and E. G. T. Liddell. 1925. Proc. Roy. Soc., xcviiiB, 589.
(4) Buytendijk, F. J. J. 1912. Zeitschr. f. Biol., lix, 35.
(5) Byrnes, C. M. 1926. Arch. Neurol. and Psychiat., xv, 407.
(6) Cooper, S. and R. S. Creed. 1927. Journ. Physiol., lxii, 273.
(7) Dusser de Barenne, J. G. 1911. Zentralbl. f. Physiol., xxv, 334.
(8) Forbes, A. and M. Cattell. 1924. This Journal, lxx, 140.
(9) Fulton, J. F. 1926. Proc. Soc. Exper. Biol. and Med., xxiii, 700.

(10) FULTON, J. F. 1926. Muscular contraction and the reflex control of movement. Baltimore: The Williams & Wilkins Co., 644 pp.
(11) FULTON, J. F. AND E. G. T. LIDDELL. 1925. Proc. Roy. Soc., xcviiiB, 577.
(12) GASSER, H. S. AND J. ERLANGER. 1927. This Journal, lxxv, 522.
(13) HEAD, H. 1920. Studies in neurology. Oxford: University Press. 2 vols., 862 pp.
(14) JOLLY, W. A. 1910. Quart. Journ. Exper. Physiol., iv, 67.
(15) LIDDELL, E. G. T. AND C. S. SHERRINGTON. 1924. Proc. Roy. Soc., xcviB, 212. 1925. Ibid., xcviiB, 267.
(16) PI-SUÑER, J. AND J. F. FULTON. 1928. This Journal, lxxxiv.
(17) PI-SUÑER, J. AND J. F. FULTON. 1928. This Journal, lxxxiii, 548.
(18) RANSON, S. W. 1925. The anatomy of the nervous system. 2nd ed., Philadelphia, Saunders, 412 pp.
(19) RAMÓN Y CAJAL, S. 1909. Histologie du Système nerveux de l'homme et les vertébrés. Paris: Maloine. 2 tom., xiv + 986 pp. (1909); 933 + [4] pp. (1911).
(20) REGAUD, C. ET FAVRE. 1904. Les terminaisons nerveuses et les organes nerveux sensitifs de l'appareil locomoteur. (Dispositifs nerveux kinesthésiques.) Lyon & Paris: A. Storck & Cie. 140 pp. First published in vol. 1 of Rev. gén. d'hist., 1904.
(21) SHERRINGTON, C. S. 1894. Journ. Physiol., xvii, 211.
(22) SHERRINGTON, C. S. 1900. The muscular sense. SHARPEY-SCHAFER'S Textbook of physiology, ii, 1002.
(23) SHERRINGTON, C. S. 1909. Quart. Journ. Exper. Physiol., ii, 109.
(24) SHERRINGTON, C. S. 1915. Brain, xxxviii, 191.

THE RESPONSE OF SMOOTH MUSCLE IN DIFFERENT IONIC ENVIRONMENTS

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Received for publication August 11, 1927

Many workers have reported experiments dealing with changes in the tone or rhythmic movements of smooth muscle immersed in solutions varying in their ionic concentration. Results obtained by such a method, however, are difficult of quantitative appraisal and have given rise to a number of contradictions in the data supplied. A few experimenters have therefore employed pharmacological agents such as epinephrine, acetyl choline and histamine as stimulants. To this group belong the experiments reported in this paper.

TECHNIC. The submaximal isotonic response of the isolated virgin guinea pig uterus to pituitary extract was obtained in various ionic environments from uterine horns which exhibited no spontaneous movements. It was thus possible to obtain good quantitative results from a chemical stimulus which acted only on muscle. Pituitary extract was made from acetone-extracted pars posterior. It was of the same strength as the international standard powder and was diluted to 1 to 10,000 parts of fresh gland by alkali-free Tyrode's solution. All dosages refer to the amounts of such a solution which were added to the 50 cc. bath in which the uterus was suspended.

All of the solutions used were prepared so that they were isotonic with, and of approximately the same ionic strength as an ultrafiltrate of blood serum. The composition of our "normal" solution and that of Tyrode's solution are given in table 1. There are considerable differences between the two solutions except in chloride and sodium ions.[1] The carbon dioxide tension in our solution was maintained at a pressure calculated to give the desired pH. The important unphysiological properties of Tyrode's solution are the high pH, the high calcium and the low bicarbonate ion concentrations, and the absence of a physiological pressure of carbon diox-

[1] The bicarbonate concentration might more accurately have been 25 millimols instead of 30 millimols per liter. Inasmuch as the carbon dioxide pressure with which the solution was equilibrated was proportionately increased the reaction was physiological and our results were uninfluenced.

ide. As will be shown later, it is impossible to work with a uterus in a solution containing as much calcium as Tyrode's solution when its pH is brought to normal by saturation with carbon dioxide at the proper tension.

The reaction of the uterus to repeated doses of pituitary extract in our normal solution was first determined. There was found, as in previous studies, a progressive increase in the reactivity of the uterus; this change was somewhat greater in our solution than in Tyrode's solution. In view of this, the response of the uterus was determined in the normal solution both before and after its response was observed in a test solution.

The normal and test solutions were prepared from freshly made isotonic stock solutions. The requisite amounts of such solutions except those of calcium chloride and magnesium chloride were measured into a cylinder and covered with oil.[2] CO_2 was admitted from the gas manifold until a pH of 7.4 was reached. This was determined by comparing samples in a

TABLE 1

Ion concentrations in millimols per liter of solution used and of Tyrode's solution

CATIONS			ANIONS		
	Solution used	Tyrode's solution		Solution used	Tyrode's solution
Na	144.0	149.0	Cl	122.0	147.0
K	6.2	3.0	HCO_3	30.0	12.0
Mg	1.0	2.0	H_2PO_4, HPO_4	1.0	0.3
Ca	0.53	1.8			
pH	7.40 ±0.05	7.88 8.00			

comparator block with standard pH tubes. The calcium chloride and magnesium chloride were then added. In the solutions no precipitation of magnesium or calcium occurred because the solubility products of the latter were not exceeded. In certain test solutions the solubility product was exceeded but no precipitation occurred during the course of the experiment. This was determined by analysis of the solution for calcium and magnesium at the conclusion of the day.

When it was desired to study the effect of decreasing the concentration of any one ion, its depletion was compensated for by increasing the sodium chloride concentration. Similarly an increase in any ion was accompanied by a decrease in sodium chloride concentration. This, of course, caused small changes in sodium chloride concentration which are without effect on the reaction of the uterus.

The presence of the indicator phenol-sulphonephthalein in the solutions was without effect on the reaction of the uterus.

[2] Throughout each experiment all test solutions as well as the bath solution were covered by a layer of pure liquid paraffin about 1 cm. thick.

METHODS. Analyses of the composition of the solutions were made as follows:

pH's were determined at 38° on the normal and test solutions by the method of Hastings and Sendroy (1924).

The CO_2 concentrations were determined when a check was desired on the Van Slyke and Neill (1924) manometric blood gas apparatus.

Frequent calcium determinations were made by the Kramer-Tisdall (1921) method.

The uterine bath was suspended in a four liter beaker filled with water which could be readily kept at 38±0.1°C. by means of the pilot flame of the Bunsen burner. Flasks containing the standard solution and one whose ion concentration differed from the standard were kept surrounded by water at 38±0.1°C. in large beakers whence the experimental solutions could be siphoned over at will into the bath. Another siphon tube from the bath to the sink permitted the removal of solutions. Two hundred and fifty cubic centimeters of each new solution were introduced into the bath in the course of replacing the 50 cc. just employed; the previously used solution was thus thoroughly removed without exposing the uterine horn to air. Bottles of twenty liters capacity were filled with air containing carbon dioxide usually at a tension of 50 mm. Hg. Such gas mixtures were forced into the solutions under examination and into the uterine bath solution by siphoning acidified water into the gas-filled bottles.

The tracings in this paper illustrate typical results of repeated observations.

RESULTS. *The effect of varying the potassium ion concentration.* Reduction in the potassium ion concentration of the solution bathing smooth muscle has been reported as causing spontaneous contraction (Tate and Clark (1922), Clark (1921), Kochmann (1925)). We have never seen a contraction of the uterus follow the reduction or even the complete removal of potassium ions. Lessening of the potassium ion concentration consistently caused diminished responses to pituitary extract similar to that shown in figure 1 A.

Mathison (1911) first reported that an increase in the potassium ion concentration causes contraction of the guinea pig uterus. This has been confirmed by many observers and apparently holds for smooth muscle in general. Usually an increase in the potassium ion concentration sufficient to alter the response of the uterus to pituitary extract initiated a series of rhythmic contractions such as are illustrated in figure 1 B. Without exception the response to pituitary extract was greater when the potassium ion concentration was raised from 6.2 to 9.3 mM. per liter of solution. With smaller increases in potassium ion concentration there was occasionally no alteration in the reactivity of the uterus Our findings are therefore contrary to those of Turolt (1922) who found that the addition of potassium

chloride (about 3.0 mM. per liter) to the bath solution inhibits spontaneous movements or causes a fall in the tone of the isolated guinea pig uterus.

The effect of varying the magnesium ion concentration. Jendrassik (1924) reported that in the presence of potassium and calcium ions an increase in the magnesium ion concentration tended to paralyze intestinal strips, while a diminution acted oppositely. Gellhorn (1926) noted that mag-

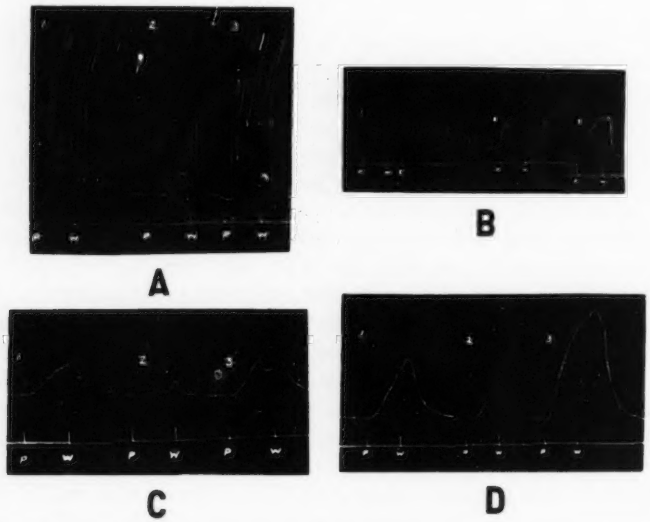

Fig. 1. Effects of altering potassium and magnesium ion concentrations. *P*, pituitary extract added to bath; *W*, washing with same solution in bath at the time the extract was given; *C*, change from standard solution to solution of altered ion concentration; pH in all instances except *D* 2, 7.40 ± 0.02.

A. Dose 0.1 cc. *1* and *3*, potassium 6.2 mM. per L. *2*, Potassium 4.6 mM. per L.

B. *1* and *3*, potassium 6.2 mM. per L. Dose 0.25 cc. *2*, potassium 9.3 mM. per L. Dose 0.2 cc.

C. Dose 0.4 cc. *1* and *3*, magnesium 1.0 mM. per L. *2*, magnesium absent.

D. Dose 0.4 cc. *1* and *3*, magnesium 1.0 mM. per L. *2*, magnesium 3.0 mM. per L. Little effect observed from smaller increases. pH 7.33.

nesium brings about a relaxation of isolated pieces of artery. Like most workers we have found that the relative concentrations of magnesium ions must be varied even more than those of potassium ions in order to elicit differences. In figure 1 C it is seen that the removal of the magnesium ions was accompanied by a diminished response to pituitary extract. A similar decreased excitability to the extract was observed when the magnesium ion concentration was increased (fig. 1 D). Magnesium is peculiar

among the ions studied in that the concentration used seemed to be optimum for eliciting a response to pituitary extract.

The effect of varying the calcium ion concentration. The question of calcium ion activity in biological fluids has interested many investigators and various methods have been employed to evaluate its magnitude.

Rona and Takahashi (1913) presented the formula

$$\frac{(Ca^{++}) \times (HCO_3^-)}{(H^+)} = K$$

as expressing the relation between the hydrogen and bicarbonate ion concentrations and the calcium ion concentration. This formula is merely another way of stating that the product $(Ca^{++}) \times (CO_3^{=}) = K_{sp}$, and is valid only in systems in equilibrium with the solid phase $CaCO_3$. It states that if the hydrogen and bicarbonate ion concentrations remain constant, the calcium ion concentration must be constant. Suppose that to a solution in equilibrium with solid $CaCO_3$ one adds a slight excess of calcium salt, keeping the bicarbonate and hydrogen ion concentrations constant. In such a solution, now supersaturated with respect to $CaCO_3$, what is the calcium ion concentration? Is all of the calcium ionized or is part of it combined with CO_3 as $CaCO_3$ not yet precipitated? Direct determination of calcium ion activity with a calcium amalgam electrode is impossible in such a solution, due to the large excess of other cations present. Furthermore the increase in concentration would be too slight to detect by conductivity measurements.

Our experiments with the guinea pig uterus have, we believe, answered the question. Changes in the response of the uterus to constant doses of pituitary extract when placed in solutions in which all of the ion concentrations, except calcium, have been constant, have been interpreted as indicating differences in calcium ion concentration in the solutions. In a sense the uterus has been a biological calcium electrode.

The effect of lowering the calcium concentration. The basic solution with which all changes are compared is one which is in equilibrium with solid $CaCO_3$. Studies made by Hastings, Murray and Sendroy (1927) have shown that the solubility product constant of $CaCO_3$ at 38° in salt solutions whose ionic strength is 0.160 is $10^{-7.40}$. This is the product of $(Ca^{++}) \times (CO_3^{=})$ in our basic solution where the calcium concentration is 0.53 millimol per liter, the pH 7.40, and the bicarbonate concentration 30 millimols per liter. When solutions are used which have the same bicarbonate and hydrogen ion concentrations as the basic solution but whose calcium concentration is 0.32 mM. per liter instead of 0.53 mM. per liter, the response of the uterus to a given dose of pituitary extract is markedly reduced. Since there is no possibility of $CaCO_3$ or a non-ionized form of calcium being formed in these solutions we interpret the diminution in

response as resulting from a diminished calcium ion concentration in the solution (fig. 2 A). When the calcium of the solution was entirely removed, almost no response of the uterus resulted from the dose of pituitary extract employed (fig. 2 B).

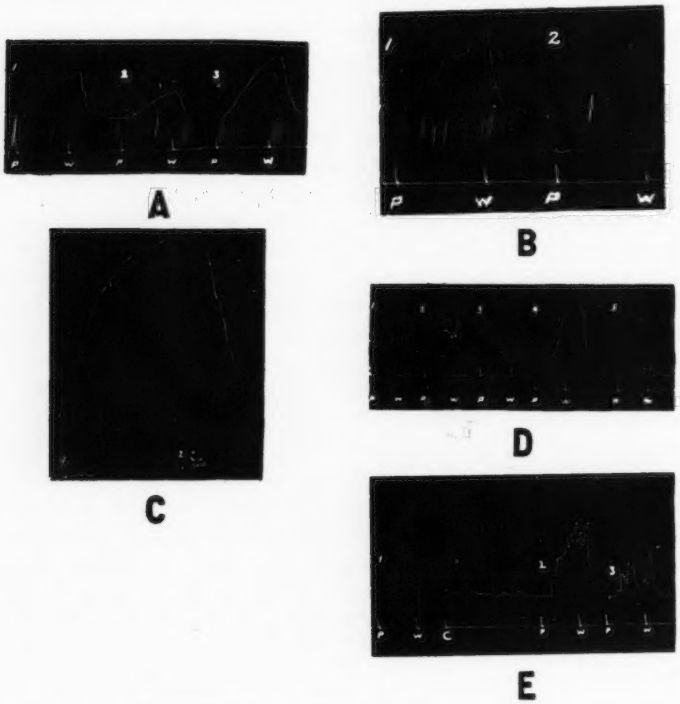

Fig. 2. Effects of alterations in calcium and phosphate ion concentrations. Lettering as in figure 1, pH's 7.40 ± 0.02 except A which was 7.47 with each test solution.

A. Dose 0.4 cc. *1* and *3*, calcium 0.53 mM. per L. *2*, calcium 0.32 mM. per L.

B. Dose 0.25 cc. *1*, calcium 0.53 mM. per L. *2*, calcium absent.

C. No pituitary extract given. *1*, calcium changed from 0.53 mM. to 1.06 mM. per L. *2*, solid $CaCO_3$ added.

D. Dose 0.1 cc. *1*, *3* and *5*, calcium 0.53 mM. per L. No phosphates. *2*, calcium 0.69 mM. per L. without phosphates. *4*, calcium 0.69 mM. per L. with 1.0 mM. phosphates per L.

E. Dose 0.35 cc. *1* and *3*, standard solution without phosphates. *2*, phosphates, 3.0 mM. per L., also present.

These experiments demonstrate the sensitivity of the uterus to slight alterations in the calcium ion concentrations of the environment. The changes in the reactivity of the uterus when calcium concentrations were

diminished or increased were qualitatively the same as those reported by many other workers experimenting with different phases of smooth muscle physiology.[3]

The effect of raising the calcium concentration. When solutions were used in which the calcium concentration approximated that of Tyrode's solution, 1.0 to 1.5 mM. per liter, the uterus contracted spontaneously and could be relaxed only in case solid $CaCO_3$ was added to the solution, in which case the excess calcium was precipitated to the equilibrium value of 0.5 mM. (fig. 2 C). This spontaneous contraction is not encountered when Tyrode's solution is used because of its high pH, 7.88 to 8.00, which diminishes the sensitivity of the uterus. This is demonstrated in figure 3 A. The solutions of such a high calcium concentration at a physiological pH irreversibly changed the uterus so that it could not be used again even when the high calcium solution was replaced by the normal solution.[4]

Experiments were then performed using solutions whose calcium concentrations were but slightly increased above the basic concentration of 0.53 mM. per liter. For example, a solution whose calcium concentration was 0.69 mM. instead of 0.53 mM. per liter gave a marked increase in response (fig. 2 D). Indeed, when the calcium concentration was raised to only 0.63 millimol per liter the increase in response was quite definite. Since these solutions with increased calcium concentrations were supersaturated with respect to $CaCO_3$ and yet the uterus reacted with an increased response to pituitary extract we feel justified in concluding that the calcium ion concentration of these solutions was increased.

To satisfy ourselves that the increased response in solutions supersaturated with respect to $CaCO_3$ was truly due to increased calcium ion concentration, the following experiment was performed.

Four solutions were prepared as follows:

	A	B	C	D
Calcium mM/liter	0.53	0.74	0.53	0.74
HCO_3 mM/liter	30.0	30.0	15.0	15.0
pH	7.40	7.40	7.40	7.40

Solution A is in equilibrium with solid $CaCO_3$.
Solutions C and D are undersaturated with $CaCO_3$.
Solution B is supersaturated with $CaCO_3$.
Other ions except phosphate ions were present as in the basic solution.

[3] Höber and his pupils have suggested that the calcium sensitivity of smooth muscle may be related to the presence of nerve plexuses. Pituitary extract probably acts directly on the smooth muscle; moreover there appear to be no ganglion cells in the horns of the guinea pig uterus.

[4] Zondek (1922) used calcium (1.8 to 5.3 mM. per liter) in amounts greatly in excess of those employed by us and observed a *relaxation* of the non-gravid guinea-pig uterus.

The response of the uterus to pituitary extract when in solutions A and B was first determined, its increased response when solution D was substituted for solution C was then noted, and finally its response in A and then in B were again observed. When allowance was made for the spontaneous increase in sensitivity of the uterus and for the diminished carbonate ion concentration (*vide infra*), it was found that the increased response in C and D was no different from the increased response in A and B. These results, we believe, clinch the fact that the calcium in solution B, although supersaturated with respect to $CaCO_3$, was in ionic form.

It should be emphasized that there were no phosphate ions in most of the solutions used in studying the effect of calcium and our results are not complicated by the effect of supersaturation with respect to $CaHPO_4$ or $Ca_3(PO_4)_2$.

The effect of varying the phosphate concentration. When one further complicates the solution by the addition of phosphates, one encounters difficulties. The response elicited when the uterus is suspended in a solution containing phosphates in a 1.0 mM. per liter concentration was little different from that obtained in the basic solution (fig. 2 D). This might be interpreted as indicating that the phosphate ions exerted no influence upon the response of the uterus. However, when the phosphate concentration was increased to 3.0 mM. per liter there was a definite increase in the response of the uterus to pituitary extract (fig. 2 E).[5] These results are difficult to interpret, except on the following basis. It would appear from the results obtained with 3.0 mM. of phosphate per liter that phosphate ions per se cause a heightened response of the uterus to pituitrin. Now all of the solutions containing phosphate were supersaturated with respect to $CaHPO_4$ and $Ca_3(PO_4)_2$. It has been shown in the papers of Hastings, Murray and Sendroy (1927) that salt solutions similar to the basic solution used in this study, containing 1.0 mM. phosphate, will remain supersaturated with respect to $CaCO_3$ for as long as 42 hours. This has lately been repeated in this laboratory and found to hold good for at least three days. Whether this is to be considered a condition of delayed equilibrium, or some other phenomenon, is immaterial for the present discussion. Suffice it to say that in such solutions the calcium and carbonate ions are not free to unite and precipitate as $CaCO_3$. In other words, the calcium ion concentration in such solutions appears to be less than in similar solutions containing no phosphate. Therefore, in the absence of a stimulating effect of the phosphate ions per se, one would expect to find a diminished response of the uterus to pituitary extract when phosphate was present. Actually, however, when only 1.0 mM. of phos-

[5] In one experiment in which the phosphate concentration was increased to 2.0 mM. per liter there was consistently a diminution in the response of the uterus.

phate was present the diminution in response, due to a decrease in calcium ion concentration, was balanced by the enhanced response due to the phosphate ions. The net result of these two effects was an apparently unchanged response. Whether this explanation is the true one must await further work with other physiological systems.

The effect of variations in CO_2 tension and hydrogen, bicarbonate and carbonate ion concentrations. With the technic employed in this study it was possible to vary the hydrogen ion concentration at will by admitting mixtures of air and carbon dioxide in varying proportions. This was done with the results shown in figures 3 A and 3 B. There was in every instance a diminution in response when the pH was raised by lowering the CO_2 tension and an increase in response when the pH was lowered by raising the CO_2 tension. In these experiments the bicarbonate concentration was constant.

In other experiments the hydrogen ion concentration was varied by varying the bicarbonate concentration but keeping the CO_2 tension constant. The results of some of these experiments are given in figures 3 C and 3 D. As in the previous experiments, when the pH was increased the response of the uterus was diminished and when the pH was decreased the response was enhanced. These experiments taken by themselves would strongly suggest that changes in the reaction of the environment were responsible for the changes in response. The following experiments would throw doubt upon this explanation, however. When the pH was kept constant, but the CO_2 tension and bicarbonate concentration were increased proportionately, there was a marked diminution in response of the uterus. Conversely, when the CO_2 tension and bicarbonate concentration were decreased proportionately, the pH therefore remaining unchanged, there was an increase in response (figs. 3 E and 3 F). The results of the above experiments have been tabulated in table 2. It is evident that the changes in response may not be attributed with consistency to pH, HCO_3 concentration or CO_2 tension, but to the CO_3 ion concentration.

Following these observations, experiments were performed in which the CO_3 ion concentration was kept constant but CO_2 tension and the bicarbonate ion and hydrogen ion concentrations were varied. The response in these instances are shown in figures 3 G and 3 H. In the first instance the bicarbonate was increased 50 per cent and the CO_2 tension was increased 300 per cent. This resulted in a 58 per cent increase in hydrogen ion concentration but no change in CO_3 ion concentration. No change in response occurred when this solution was used in the bath.

In the second instance the bicarbonate was reduced 50 per cent, the CO_2 tension was reduced 75 per cent. The hydrogen ion concentration was found to be reduced 39 per cent but the CO_3 ion concentration was practically unchanged. Other similar experiments caused either no change in

response or a slight change which was at times positive and at times negative. These experiments we therefore interpret as proving the importance of the CO_3 ion per se in the particular physiological system with which this work has been concerned.

Were it not for our results upon changes in calcium concentration in the solution, one would be inclined to interpret the above correlation between the activity of the uterus and the CO_3 ion concentration as merely an indirect calcium effect. However, in view of the fact that we have

TABLE 2

The effect of variations in CO_2 tension and hydrogen, bicarbonate and carbonate ion concentrations

pH	CO_2 TENSION	HCO_3 CONCENTRATION	CO_3 CONCENTRATION	RESPONSE	FIGURE
Increased	Lowered	Unchanged	Increased	Diminished	3 A
Diminished	Raised	Unchanged	Diminished	Increased	3 B
Increased	Unchanged	Increased	Increased	Diminished	3 C
Diminished	Unchanged	Diminished	Diminished	Increased	3 D
Unchanged	Raised	Increased	Increased	Diminished	3 E
Unchanged	Lowered	Diminished	Diminished	Increased	3 F
Diminished	Raised	Increased	Unchanged	Unchanged	3 G
Increased	Lowered	Diminished	Unchanged	Unchanged	3 H

Fig. 3. Effects of changes in hydrogen, bicarbonate and carbonate ion concentrations. Lettering as in figure 1.

A. Dose 0.25 cc. *1* and *3*, air bubbled through bath solution. pH 7.76. *2*, air with CO_2 tension of 50 mm. Hg bubbled through bath solution. pH 7.42.

B. Dose 0.2 cc. *1* and *3*, CO_2 tension 50 mm. Hg. pH 7.40. *2*, CO_2 tension 100 mm. Hg. pH 7.18.

C. Dose 0.05 cc. CO_2 tension 50 mm. Hg. *1* and *3*, HCO_3 concentration 45.0 mM. per L. pH 7.60. *2*, HCO_3 concentration 30.0 mM. per L. pH 7.42.

D. Dose 0.05 cc. CO_2 tension 50 mm. Hg. *1* and *3*, HCO_3 concentration 30.0 mM. per L. pH 7.42. *2*, HCO_3 concentration 15.0 mM. per L. pH 7.32.

E. Dose 0.2 cc. *1* and *3*, CO_2 tension 50 mm. Hg. HCO_3 concentration 30.0 mM. per L. pH 7.43. *2*, CO_2 tension 100 mm. Hg. HCO_3 concentration 60.0 mM. per L. pH 7.40.

F. Dose 0.2 cc. *1* and *3*, CO_2 tension 25 mm. Hg. HCO_3 concentration 15.0 mM. per L. pH 7.47. *2*, CO_2 tension 50 mm. Hg. HCO_3 concentration 30.0 mM. per L. pH 7.44.

G. Dose 0.35 cc. *1* and *3*, HCO_3 concentration 30.0 mM. per L. CO_2 tension 50 mm. Hg. pH 7.42. *2*, HCO_3 concentration 45.0 mM. per L. CO_2 tension 150 mm. Hg. pH 7.22.

H. Dose 0.25 cc. *1* and *3*, HCO_3 concentration 30.0 mM. per L. CO_2 tension 50 mm. Hg. pH 7.42. *2*, HCO_3 concentration 15.0 mM. per L. CO_2 tension 12.5 mm. Hg. pH 7.59.

demonstrated that a solution may be supersaturated with respect to $CaCO_3$ but behave as if all of the calcium present is in ionic form, it seems unreasonable to suppose that making the solution supersaturated with respect to $CaCO_3$ by raising the CO_3 ion concentration would affect the calcium ion concentration. We are therefore inclined to attribute the changes in

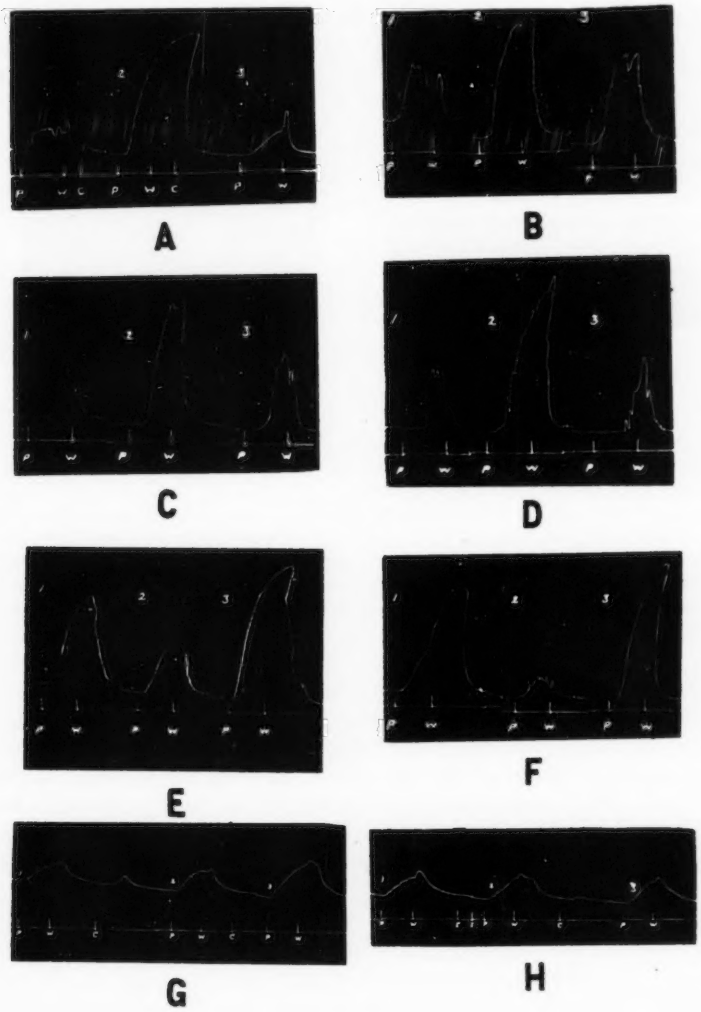

Fig. 3

the response of the uterus encountered in these experiments to changes in CO_3 ion concentration per se. This does not preclude the possibility of changes in hydrogen ion concentration having an effect of their own, particularly when they are of greater magnitude than those produced in these experiments. It has perhaps been too little appreciated that the CO_3 ion concentration is kept remarkably constant in the fluids of the organism. Calculations made by Murray and Hastings (1925) indicated that in the blood serum the CO_3 ion concentration is the most constant of all of the variables which change during a respiratory cycle. In view of this, marked sensitivity to changes in its concentration would hardly be surprising.

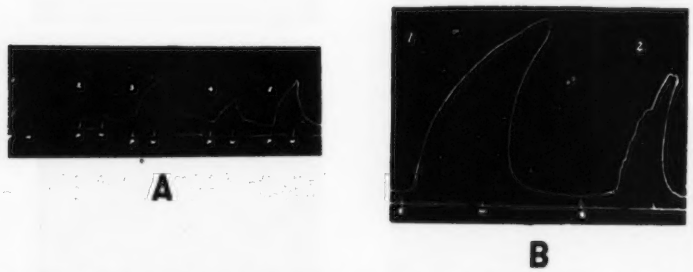

Fig. 4. The effects of varying all ions simultaneously by dilution of the standard solution with isotonic sucrose solution.

A. Dose 0.35 cc. pH in all instances 7.40 ± 0.02. *1*, *3* and *5*, standard solution. *2*, half dilution of all ions. *4*, half dilution of all ions except Na^+ and Cl^-.

B. No pituitary extract given. Air bubbled through bath solution. Washings with standard solution. *1*, sucrose solution containing phosphate buffer solution. Apparent pH 7.28. *2*, sucrose solution containing a trace of NaOH. Apparent pH 7.17.

The effect of reducing the concentration of all ions. Since Dale (1913) has pointed out how readily the response of the guinea pig uterus may be altered by slight changes in the osmotic pressure of the bath solution, great care was taken in all experiments to keep the solutions isotonic. Cane sugar is commonly held to be as satisfactory as any substance as an osmotic substitute for salts and was employed whenever a relatively large proportion of inorganic ions was experimentally replaced by a non-ionized substance.

In figure 4 A the typical effects of variations of all ions and especially those of NaCl are illustrated. Control stimulations of the uterus by pituitary extract produced the contractions numbered *1*, *3* and *5*. When all ions had been halved in a solution whose isotonicity had been preserved by cane sugar, there was almost no response by the uterus to the extract

(no. *2*). The contraction of no. *4*, which appeared in a solution all of whose ions, except those of NaCl, were reduced to one-half, was still less than those elicited in the normal solution but much greater than that of no. *2*. Increase of NaCl ions therefore clearly increased the excitability of the uterus to pituitary extract.

The effect of substituting all ions with sucrose. The effect of changing from the standard solution to one containing only isotonic cane sugar and

TABLE 3

The effect of various ionic environments on the response of the isolated guinea pig uterus to pituitary extract

ION VARIED	CONCENTRATION INCREASED (+) OR DIMINISHED (−)	RESPONSE TO PITUITARY EXTRACT INCREASED (+) OR DIMINISHED (−)	REMARKS
K^+	−	−	No spontaneous contraction
	+	+	Ion change alone initiated rhythmic contractions
Mg^{++}	−	−	Concentration of Mg used seemed optimum
	+	−	for eliciting contraction by pituitary extract
Ca^{++}	−	−	No response when all of Ca was removed
	+	+	Response equally increased whether or not Ca was supersaturated with respect to $CaCO_3$
$H_2PO_4^-$, $HPO_4^=$	−	No change	See text for discussion
	+	+	
$CO_3^=$	−	+	See text for discussion
	+	−	
Na^+, Cl^-	−	−	
	+	+	
All Ions	−	−	Complete removal provoked spontaneous contraction perhaps due to sucrose

phosphate buffer solution (apparent pH 7.28) is shown in figure 4 B. A marked contraction was produced which subsided at once when the bath solution was again replaced by the normal solution. The second contraction shown in figure 4 B appeared with the replacement of the standard solution by isotonic cane sugar made slightly alkaline (pH 7.17) by a trace of NaOH solution. Like the first contraction, it disappeared when the cane sugar solution was washed out by the normal solution. The greater

contraction in the first instance probably is to be attributed to the phosphates in the solution. A stimulation of the isolated dog auricle (Andrus, 1924) and of the frog esophagus (Stiles, 1901) has previously been observed when isotonic glucose solutions replaced physiological saline solutions. It is therefore impossible to decide from our experiments alone whether the absence of all ions or the presence of the cane sugar stimulated the uterus to the spontaneous, sustained contraction which occurred.

Discussion. It was hoped in the beginning of this work that a quantitative comparison between the magnitude of the response of the uterus to pituitary extract and the change in the ionic environment could be made. Partly due to the spontaneous increase in sensitivity and partly because of the differences between individual uteri this was not fully realized. It was possible, however, to determine approximately the minimum change in any one ion which would cause a measurable and definite change in response. The results have been collected in table 3 and, though approximate, are interesting in that they illustrate the relatively greater importance of changes in calcium ion activity than of those of any of the other ions studied. Furthermore the doubling of the calcium ion activity caused profound irreversible changes in the uterus, an effect not encountered with the other ions.

The other matter of interest was the sensitivity of the uterus to changes in carbonate ion activity. This change in response with varying carbonate ion activity seemed not to be related primarily to changes in hydrogen or calcium ion activity. It would seem that the response of the uterus in vitro is within the limits studied independent of the CO_2 tension, but dependent upon the $CO_3^=$ ion activity. This is most clearly understood by referring to the nomograph of Murray and Hastings (1925) in which the path taken by the uterine response would be identical with that labeled "kidney function."

SUMMARY

1. The response of the guinea pig uterus to pituitary extract in salt solutions of varying composition has been studied.

2. Variations in all physiologically essential ions singly or together alter the response. Changes in calcium ion activity produce the greatest relative change in response.

3. The uterus was found to be sensitive to changes in the carbonate ion activity per se. The significance of this has been discussed.

4. A physiological salt solution, normal with respect to its inorganic constituents, has been described and used.

BIBLIOGRAPHY

Andrus, E. C. 1924. Journ. Physiol., lix, 361.

Clark, A. J. 1921. Journ. Pharm. Exper. Therap., xviii, 423.

DALE, H. H. 1913. Journ. Physiol., xlvi, xix.
GELLHORN, E. 1926. Pflüger's Arch., ccxiii, 789.
HASTINGS, A. B., C. D. MURRAY AND J. SENDROY, JR. 1927. Journ. Biol. Chem., lxxi, 723.
HASTINGS, A. B. AND J. SENDROY, JR. 1924. Journ. Biol. Chem., lxi, 695.
JENDRASSIK, L. 1924. Biochem. Zeitschr., cxlviii, 116.
KOCHMANN, M. 1925. Biochem. Zeitschr., clxi, 390.
KRAMER, B. AND F. F. TISDALL. 1921. Journ. Biol. Chem., xlvii, 475.
MATHISON, G. C. 1911. Journ. Physiol., xlii, 471.
MURRAY, C. D. AND A. B. HASTINGS. 1925. Journ. Biol. Chem., lxv, 265.
RONA, P. AND D. TAKAHASHI. 1913. Biochem. Zeitschr., xlix, 370.
STILES, P. G. 1901. This Journal, v, 338.
TATE, G. AND A. J. CLARK. 1922. Arch. internat. d. pharmacodyn. et de therap., xxvi, 103.
TUROLT, M. 1922. Arch. Gynaekol., cxv, 600.
VAN SLYKE, D. D. AND J. M. NEILL. 1924. Journ. Biol. Chem., lxi, 523.
ZONDEK, S. G. 1922. Biochem. Zeitschr., cxxxii, 362.

THE BILE-EXPELLING MECHANISM OF THE GUINEA PIG

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Received for publication August 22, 1927

The question of the relative importance of the gall bladder musculature in connection with the emptying of that organ has been the subject of a number of recent papers; and, considering that there are still two completely divergent schools of thought, it may be taken that the matter is far from settled. It was suggested to one of us (G. E. B.) who does not believe in the expulsion of bile by the active contraction of the gall bladder musculature, by two supporters of that view (Drs. F. C. Mann and G. M. Higgins) that a glance at the gall bladder of a recently killed guinea pig would afford convincing proof of the active contraction of that organ. After looking at the gall bladder, this observer, who has obtained evidence in the dog of the importance of duodenal peristalsis as a factor in emptying the biliary system (Burget, 1925, 1926, 1927), naturally looked downwards to the duodenum in order to note whether any correlation existed in the guinea pig between peristalsis of the gut and shrinking of the gall bladder; but his attention was here attracted by an unique structural arrangement in what at first appeared to be a dilatation of the common bile duct before its passage through the duodenal wall; further, this dilatation was observed to contract at intervals. Westphal (1923) studied the activity of the bile passages in the guinea pig and described the gross appearance of the dilated area lying on the duodenum. His conception of the activity of the lower end of the common duct is slightly at variance with our findings as will be pointed out below. With the exception of Westphal's brief gross description, this mechanism has remained undescribed in all the literature which deals with the guinea pig's biliary system (Reach, 1919; Mann, 1920; Mann and Brimhall, 1920; Higgins and Mann, 1926; Mann and Higgins, 1927a) in spite of the fact that measurements and even drawings have been published. We have recently made a preliminary report (1927) on the guinea pig's bile-expelling mechanism, and Mann and Higgins (1927b) have also made mention of it. The present paper endorses, amplifies, and in some points, modifies the findings given in our preliminary report.

Anatomy. The extra-hepatic portion of the biliary system in the guinea pig consists of hepatic ducts, cystic duct, gall bladder, common bile duct,

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and a contractile ampulla which opens into the duodenum. The anatomy of this ampulla requires special mention as it is apparently peculiar to the guinea pig and is of great functional significance in the expulsion of bile. It is a hollow structure, holding when distended as much as 0.2 cc. of bile, applied to the antero-superior aspect of the duodenum, 4 to 8 mm. from the pylorus; the first part of the guinea pig's duodenum forms a well-marked anatomical cap and is frequently of twice the diameter of the remainder. Bile flowing down the common duct enters a small dilatation where the duct is doubled on itself, and then passes directly into the proximal end of the ampulla (see fig. 1). Piercing the wall common to ampulla and duodenum 1 to 2 mm. from the distal end of the former, is a short direct tube which starts as a shallow funnel-shaped depression in the ampulla and opens

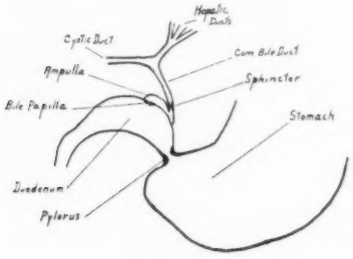

Fig. 1

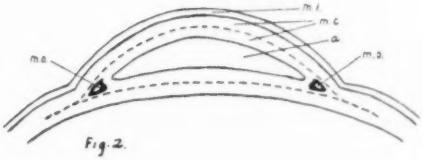

Fig. 2.

Fig. 1. Diagram to show locations of ampulla, papilla and sphincter.

Fig. 2. Diagram to show the arrangement of plain muscle in the bile ampulla. The dotted lines indicate the distinction between the muscle proper to the ampulla and that resulting from a splitting of the duodenal muscularis, *m. o.*, ring of muscle fibers, frequently oblique.

at the heal of a papilla in the duodenum. The pancreatic duct is entirely separate and opens several millimeters distal to the bile papilla.

Histology. By means of appropriately stained sections, the following histological details have been made out (see fig. 3).

The *gall-bladder* is practically always free from the liver, being connected to the duodenum below by a kind of mesentery. It is thus completely covered by a peritoneal coat, beneath which is a subserosa of considerable thickness, containing many blood and lymph vessels embedded in a fairly loose connective tissue; the latter contains a few elastic fibres disposed principally just beneath the serosa.

The muscularis consists of two main layers of plain muscle: the outer, longitudinal, compact, with a tendency to incompleteness and a bundle arrangement; the inner, circular, complete, usually thinner than the longitudinal, but with the fibres more separated by connective tissue. Occa-

sionally there are seen, within the circular muscle, a few small isolated bundles of fibres, forming an innermost longitudinal layer. Under certain conditions when the muscle is contracting, definite diverticula appear in the wall of the gall bladder, looking from without like small spherical buds, 0.5 to 2.0 mm. in diameter. It would appear that these diverticula originate in the weaker parts of the gall bladder, as their walls are usually thinner than the rest and contain less muscle, sometimes none at all. At the base of the attachment of each diverticulum the muscle (particularly longitudinal) is thicker than elsewhere (fig. 3, *1*).

The mucosa consists of a tunica propria of loose connective tissue with very few elastic fibres, and is lined by a columnar epithelium; like the subserosa it contains rich plexuses of blood and lymph vessels (the extreme vascularity of the organ can easily be seen with the naked eye). The mucous membrane is much flattened in the distended gall bladder, but in the undistended condition it is formed into large irregular folds; in some of the latter preparations the tunica propria appears especially loose and even evacuolated. There are no mucosal glands, but a few goblet cells are seen in the epithelium.

At the neck of the gall bladder (fig. 3, *2*) the mucous membrane becomes much simpler, though a few large tubes are to be seen (ducts of Luschka?). The muscle coat becomes much thinner and is incomplete. The subserosa remains a thick layer and contains many large lymphatics; both it and the tunica propria of the mucosa contain rather more elastic tissue than they do in the fundus. In this region also there is a kind of short spiral valve which somewhat narrows the lumen locally.

The *cystic duct* and the *hepatic ducts* require little mention, except that they usually appear to contain no muscle.

Fig. 3. *1. Fundus of gall bladder, transverse section.* (×17). *s*, serosa; *s.s.*, subserosa; *m*, muscularis, longitudinal and circular; *mm.*, mucous membrane; *d*, incipient diverticulum (note almost complete absence of muscle in its wall, but thickening of its muscle at its rim, particularly the longitudinal fibres on the upper side).

2. Neck of gall bladder, transverse section. (×42). *l*, one of many large lymphatics; *d-L*, duct of Luschka. Other lettering as in *1*.

3. Transverse section through common bile duct, ampulla and sphincter. (×17). *a*, ampulla; *d*, duodenum; *c*, *b*, *d*, common bile duct; *sph.*, sphincter. (A poor section but the best available for photographing.)

4. Distal half of bile ampulla, longitudinal section (×20) (ampulla and duodenum distended). *d, duodenum; a*, ampulla; *b. v.*, blood vessel.

5. Proximal half of ampulla, transverse section. (×18) *m.c.*, circular muscle fibres; *m.l.*, longitudinal muscle fibres.

6. Transverse section of ampulla, showing point of entrance into duodenum. (×18). *p*, bile papilla; *m.p.*, muscle in papilla; *dt*, duct.

7. Transverse section of ampulla, just distal to center of bile papilla. (×18). The muscular duodeno-ampullary wall has just been restored after having given passage to the duct.

The *common bile duct* is similar to the other ducts except that in its distal 2 mm. a thick and compact circular coat of plain muscle is present (fig. 3, *3*); correlating the physiology (see below) and the histology of this part,

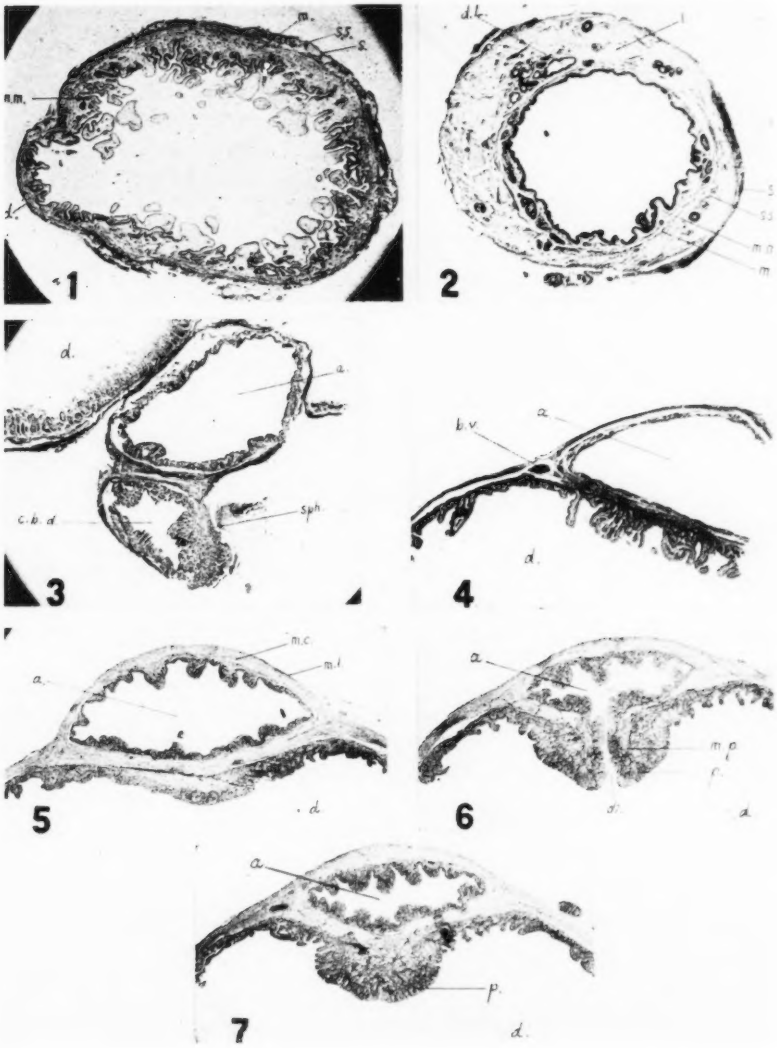

Fig. 3

it is reasonable to look on this muscle as a definite sphincter, and the name "sphincter" throughout the rest of the paper refers, unless otherwise stated, to this musculature. The mucous membrane in this region becomes raised into folds; it contains no glands, but there are goblet cells in the epithelium. Just distal to the sphincter the common duct dilates, having rather less muscle and a thinner mucosa, and after a sharp turn it enters the ampulla.

The *ampulla* (fig. 3, 4 and 5), as previously described, lies not unlike a cap on the right anterior border of the duodenum. The serous coat is continued directly from one to the other. It has a thick and compact muscularis (fig. 2), consisting of an outer longitudinal and an inner circular layer; the former is directly continuous with the longitudinal layer of duodenal muscle, but it is decidedly thinner. The circular coat is about five times as thick as the longitudinal layer, but is of about the same thickness as the circular coat of the duodenum. The circular muscle coats of both ampulla and duodenum split into halves at the edges of the ampulla, the inner half of each blending to form a muscular partition common to both cavities. The small triangular space left at the point of splitting is filled by a bundle of muscle fibres running longitudinally or slightly obliquely. Between the two muscle coats are numerous small ganglia of nerve cells, corresponding undoubtedly to Auerbach's plexus. In some preparations these ganglia have been seen at the junctional edge of ampulla and duodenum.

The mucous membrane of the ampulla is irregular in thickness, but is not as thick as that of the duodenum; it becomes folded when the ampulla is contracted. At its base are a number of short curved tubular glands (mucous), and there are a number of goblet cells in the epithelium though these are not as frequent as in the duodenum.

Close (1 to 2 mm.) to the distal end of the ampulla the floor contains a small shallow depression; from the apex of this "funnel" a duct, lined by a mucous membrane, leads directly into the duodenum by passing at right angles through the wall. The duct terminates at the head of a very definite papilla which is formed very largely of thickened duodenal mucosa (fig. 3, 6 and 7). The common muscular floor is, of course, incomplete at this point and passes around into the papilla around the duct, thinning out as it does. Toward the termination of the muscle layer in the substance of the papilla the fibres change their direction and become arranged circularly around the duct, i.e., sphincter-wise; but it is noticeable that this "sphincter," which is possibly homologous with that commonly described as Oddi's sphincter in other animals, consists, not of a thickened aggregation of muscle fibres, but rather of a thinning out of an existing muscle layer. In animals such as the dog, however, the sphincter of Oddi lies in the duodenal submucosa and appears to be a thickening of the muscularis mucosae;

whereas in the guinea pig this "sphincter" seems to be derived directly from the *true* muscularis.

From this account it can be seen that the ampulla is really an integral part of the duodenum, the peritoneal and longitudinal muscle coats being actually common to both cavities, such that the ampulla is partially embedded in the duodenal wall. This relationship is further strengthened by observing its actual mechanism.

Physiology. In Westphal's investigation of the activity of the bile passages of the guinea pig, he describes the structure which we have called the ampulla, as an oblong somewhat elevated area about 6 mm. by 3 mm., lying on the posterior wall of the duodenum. The great contractility of this region of the common duct and the extreme blanching that accompanies contraction were noted by him. It appeared to him to be a peristaltic wave originating near the middle of the common duct and becoming more vigorous as it approached the duodenum. He observed that this activity of the ductus choledochus took place simultaneously with a rapid duodenal peristaltic wave. Its effect upon emptying the gall bladder he states as follows: "Die Gallenblase, die vorher eine Stunde lang ihre Grösse unverändert beibehalten hatte, wird innerhalb 6 Minuten bei dieser lebhaften Peristaltik am unteren Choledochus sehr klein, erbsengross, 6:5:4 mm., weiss und undurchsichtig in ihrer Wand."

We have studied the functioning of these structures following exposure by making a midline incision and turning up the liver. In most of the experiments barbital anesthesia was used; in some, ether; and in a few instances the exposure was made with the use of a local anesthetic only. The earlier observations were made immediately after the death of the animal by a sharp blow on the back of the head. Further study was made in the decerebrate animal after allowing 45 minutes to one hour for recovery from shock; artificial respiration was used and at the time of observation most of the anesthetic (ether) had been thrown off. We have also studied the biliary passages following cholecystectomy. In all instances the bile passages upon exposure have been found distended with bile and their walls translucent.

The activity of the sphincter and ampulla is very marked following sudden death of the animal when asphyxia is bringing about contractions of the gastro-intestinal tract. Either with the approach of a peristaltic wave of the duodenum or at the time of a strong contraction of the duodenal cap, the sphincter may be seen to contract tightly and become quite blanched. Thus the lumen of the common duct above the ampulla is obstructed. Contraction of the ampulla at once follows, expelling its contents into the duodenum, the sphincter remaining contracted throughout. Both sphincter and ampulla now relax and bile flows into the latter until it is distended; the time necessary for filling depends upon the tension

in the gall bladder and in the hepatic ducts. At the instance of another strong contraction of the duodenum the cycle of events is repeated and bile is again expelled into the duodenum. The activity of these structures resulting from asphyxia following sudden death of the animal usually results in emptying the gall bladder in 5 to 8 minutes. Tonus changes and weak contractions of the duodenum are not effective in bringing about activity of this bile expelling mechanism.

Similar activity of the sphincter and ampulla was seen to take place during digestive peristalsis of the duodenum when exposure had been made with the aid of a local anesthetic. Less vigorous but, nevertheless, distinct activity was sometimes observed when a general anesthetic was used. These contractions were not so strong and by no means so frequent as those produced by asphyxia.

The sphincter seems to be a highly irritable structure. It has often been seen to undergo rhythmic contractions at a rate of 10 to 15 per minute. When quiescent, the slightest mechanical stimulation such as touching it or stretching the common duct elicits prompt contraction. Stimulation with a very weak faradic current is always adequate. Stronger stimulation causes contraction of the ampulla as well.

The ampulla, also, responds readily to either mechanical or faradic stimulation by strongly contracting, being only slightly less irritable than the sphincter; such contraction is accompanied by contraction of the sphincter. The filled gall bladder of an anesthetized animal may be emptied in a few minutes by repeatedly stimulating the ampulla with a faradic current allowing intervals for its relaxation and filling. Similarly when the cystic duct is cannulized and connected with a vessel containing normal saline, this fluid can be rapidly expelled into the duodenum. This constitutes ample proof of its effectiveness as a bile-expelling mechanism. Although our earlier observations on the effect of distending the ampulla by pressing on the partially filled gall bladder led us to believe that it would not respond by contracting, further investigation has shown that under favorable conditions such distention is usually followed by contraction of both sphincter and ampulla. In this respect there is a resemblance to the reaction of the intestine following mechanical distention. To what extent, if at all, the flow of bile is normally regulated by the tension in the ampulla would be difficult to establish. That distention to a point beyond normal will produce contraction makes for an efficient bile pressure regulating mechanism and would prevent over-distention of the gall bladder.

While observing the exposed gall bladder, intact and distended with bile, it has been seen to undergo slight changes in shape. There can be no doubt but that these changes in shape were due to muscular contraction and relaxation of the nature of tonus changes. That the gall bladder of the guinea pig cannot undergo marked changes in size by the contraction

of its musculature upon direct stimulation is shown when the viscus is exposed under local anesthesia and the cystic duct cannulized without allowing loss of tension in the organ. The cannula is connected with a one cubic centimeter graduated pipette containing physiological saline. The tension can be approximately estimated and there is only slight change in the volume of the viscus by a small error in this estimation. If, now, the gall bladder is stimulated with a medium faradic current in two or three places a rise of 5 to 9 mm. of pressure in the pipette may be effected. For example, the existing pressure in a gall bladder was 89 mm. water; following stimulation it was raised to 98 mm. These figures represent the highest range obtained in our experiments and depict feeble muscular power on the part of the organ when stimulated.

Halpert (1927) has recently made the suggestion that the muscularis of the gall bladder may be homologous with the muscularis mucosae of the duodenum. He gives evidence to support this hypothesis. We find that stimulation of the gall bladder in the guinea pig elicits only a local response. In the investigation of the intestinal mucosal motor mechanism, King and Arnold (1922) found that its response to stimulation was local in character. In this regard, then, our findings may be considered as favoring Halpert's views.

After killing an animal quickly with chloroform, Mann and Higgins (1927b) exposed the gall bladder and stimulated it. The viscus was noticed to grow rapidly smaller in size during the next few minutes. They cite this as evidence that the gall bladder of the guinea pig has marked contractile properties and that it expels bile by the force of its muscular contraction. The latter conclusion does not seem to be warranted by the evidence. In experiments of this type it is not necessary to stimulate the gall bladder in any way in order to see it grow smaller in size. It will do so even though the greatest care is taken to prevent its being stimulated. The repeated contractions of the ampulla, initiated by asphyxial contractions of the duodenum tend to empty the organ of its contents and it retracts in size by a process probably largely passive, i.e., by changes in tone.

The best preparation for studying the effect of vagus stimulation on the biliary passages is the decerebrate animal. Under ether anesthesia a tracheal cannula was inserted and the vagi isolated. The cord was then transected below the medulla and artificial respiration supplied. The brain was pithed and the animal covered and allowed to remain unmolested for 45 minutes to one hour, at the end of which time the bile passages were exposed in the usual manner. They were always distended with bile and the stomach and duodenum were quiescent. Following stimulation of the vagus with a medium faradic current, strong peristaltic movements of the stomach are precipitated. After a latent period of one or two minutes duodenal contractions are also manifested and, with these, activity of the

sphincter and ampulla. These movements are similar to those seen following sudden death of the animal and may be due in part to the asphyxia caused by vagus inhibition of the heart. The gall bladder may be emptied of its contents in a few minutes under these conditions. Intravenous injection of pilocarpine or eserin tends to produce similar effects by causing activity of the duodenum.

In order to investigate the part this organ might play in emptying its contents in these experiments by actively contracting, a small curved glass cannula was inserted into the common duct by way of the duodenum, papilla duct, and ampulla. One end of the cannula lay in the common duct and the other projected outside the duodenum where it was connected with a graduated 1 cc. pipette filled with saline solution. The cannula fit closely enough to remain in place and prevent loss of fluid without a ligature. Nerve fibres to the gall bladder were left intact by this procedure. The pressure varied in different experiments but was usually between 60 and 70 mm. water; the passage was free between the gall bladder and pipette. Stimulation of the vagi, now, did not cause any perceptible change in the pressure in the pipette, indicating that the gall bladder musculature did not respond although strong contractions of the stomach were induced. We are thus led to believe that in the emptying of the gall-bladder under the above experimental conditions, active muscular contraction of the organ plays only a minor rôle, the activity of the sphincter and ampulla constituting the more important expelling force.

Although Westphal (1923) does not give the details of preparing his animals, this is probably the type of preparation used by him. As regards activity of the involved structures following vagus stimulation we confirm most of his observations. However, as regards the nature of the activity in the common duct he failed to make the correct interpretation. Westphal also claims to have seen peristalsis in the cystic duct. We have never seen this and have failed to find much musculature in the wall of this duct. As to what extent he believed the gall bladder aided in the expulsion of bile in his experiments on the guinea pig, he fails to make clear.

The effect of cholecystectomy was studied in seven animals. The gall bladder was removed under ether anesthesia and aseptic conditions. It has only a slight attachment to the liver and consequently is very easily removed. All these animals made a rapid recovery and were in excellent condition when brought to autopsy. The length of time which elapsed from the cholecystectomy to autopsy ranged from 9 to 48 days. The cystic duct stump varied in length from 0.2 cm. to 1.5 cm. In no case was there found any marked dilatation of any of the ducts. Moderate dilatation occurred in two animals which had post-operative periods of 24 days and 33 days. The remaining five showed no dilatation. We have seen dilatation in normal animals as great as was shown in the two animals mentioned.

Therefore, no particular significance can be attached to this finding. In all cases the sphincter and ampulla were normal in appearance and function.

The series is not large enough, nor the post-operative period sufficiently long, to warrant drawing definite conclusions. However, that there was not more than a very slight indication of dilatation of the ducts is suggestive of a compensation on the part of the remaining mechanism for whatever function of pressure adjustment the gall bladder may have exercised. That is to say, when the ampulla became over-distended by increased biliary pressure due to the lack of storage and absorption by a gall bladder, it may have responded by contracting and expelling its contents. One other possibility would seem to exist, namely, the sphincter in the papilla may have yielded to the increased pressure and thus allowed a more or less continuous flow. However, if the latter were true, with a normal resistance at the papilla, it would seem that some dilatation should have been found in the biliary ducts of all cholecystectomized animals. The ducts were in some cases well distended with bile but after one or two contractions of the ampulla they returned to normal size. It is worthy of note that only one animal of the series had diarrhea after the operation, the feces of the others being normal.

The musculature of the papilla may conceivably have the same function as is claimed for Oddi's sphincter in some other animals. But considering that the muscle is somewhat thinned out, and not thickened, in the papilla it seems that the papilla as a whole probably acts as a non-return valve, the thick mass of mucous membrane forming a soft and effective plug. We have not been able, by pressure, to force duodenal contents into the intact ampulla.

SUMMARY AND CONCLUSIONS

That the guinea pig possesses a specialized form of bile-expelling mechanism has been shown by this investigation. An ampulla, lying on the duodenum, by active contraction discharges its contents directly into the duodenum when the latter undergoes a strong contraction or when a peristaltic wave traverses it; regurgitation into the common duct is prevented by the simultaneous contraction of a strong sphincter. The anatomical arrangement described would seem to be a variation of the usual oblique course of the intramural portion of the common duct in most other mammals. It is possible that the ampulla, though lying practically outside the duodenal musculature, is in reality homologous with the true ampulla of Vater.

These observations are of considerable interest for two reasons: 1, while the gall bladder of the guinea pig possesses contractile properties, the flow of bile is probably only slightly, if at all, dependent upon its powers of contraction; 2, the close relationship of this bile-expelling mechanism

to activity and peristalsis of the duodenum should stimulate further investigation as to a similar relationship in other mammals between the intramural portion of the common duct and peristalsis of the duodenum.

BIBLIOGRAPHY

BURGET, G. E. 1925. This Journal, lxxiv, 583.
1926. This Journal, lxxix, 130.
1927. This Journal, lxxxi, 422.
BURGET, G. E. AND R. J. BROCKLEHURST. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 843.
HALPERT, B. 1927. Johns Hopkins Hosp. Bull., xl, 390.
HIGGINS, G. M. AND F. C. MANN. 1926. This Journal, lxxviii, 339.
KING, C. E. AND L. ARNOLD. 1922. This Journal, lix, 97.
MANN, F. C. 1920. Anat. Record, xviii, 355.
MANN, F. C. AND G. M. HIGGINS. 1926a. Proc. Soc. Exper. Biol. and Med., xxiv, 533.
1926b. Proc. Soc. Exper. Biol. and Med., xxiv, 931.
REACH, F. 1919. Arch. f. exper. Path. u. Pharm., lxxxv, 178.
WESTPHAL, K. 1923. Zeitschr. f. klin. Med., lxliv, 22.

A DIETARY DEFICIENCY CANINE DISEASE—FURTHER EXPERIMENTS ON THE DISEASED CONDITION IN DOGS DESCRIBED AS PELLAGRA-LIKE BY CHITTENDEN AND UNDERHILL AND POSSIBLY RELATED TO SO-CALLED BLACK TONGUE

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Received for publication September 24, 1927

PART I. EXPERIMENTS ON THE PREVENTION OF THE DISEASE. In 1917 Chittenden and Underhill (1) called attention to the production in dogs of a pathological condition which closely resembles human pellagra.

The disease may be induced in dogs by the ingestion of a diet containing boiled peas as the chief source of nitrogen. Under suitable conditions these nutritional disturbances may be manifested even when raw meat is included in the diet. By quantitative variations in the food intake the condition may be produced in varying periods of time ranging from one month to six or eight months. The onset of the pathological symptoms is generally very sudden. Usually the first abnormal manifestation is refusal to eat, and examination will reveal nothing to account for the loss of appetite. The animal lies quietly in its pen and is apathetic. After continued refusal to eat for a day or two, the mouth of the dog will present a peculiar and characteristic appearance. The inner surface of the cheeks and lips and the edges of the tongue are so covered with pustules as to give the impression of a mass of rotten flesh. The odor from these tissues is foul and almost unbearable. When stroked with absorbent cotton the mucous lining of the mouth comes away in shreds. Intense salivation is present. The teeth appear solid and normal. A bloody diarrhea is present, attempts at defecation being very frequent and resulting in the passage of little more than a bloody fluid of foul odor. In some cases, the thorax and upper part of the abdomen may contain many pustules half an inch in diameter which are filled with pus organisms. No other skin lesions are prominent. Death usually results without any particularly striking features.

Certain dogs presented symptoms which were not common to all but undoubtedly bear a relationship to the usual manifestations. One animal apparently in the best of health was seized with a convulsion and died in a short time. Another developed the same type of convulsions but lingered for a period of a day, during which convulsions occurred at the rate of one every five or six minutes. In the earlier part of this period the animal exhibited a blind, staggering gait and would run her nose into a wall as though she could not see it. The left eye was closed and twitching, and the left legs were not under complete control. The right side seemed normal. During the latter part of the day the dog lay in a state that appeared to be a mild continuous spasm. A third dog showed a single convulsion as

the initial symptom, and on the following day the characteristic foul mouth and bloody diarrhea were in evidence.

At autopsy, two types of conditions are recognizable. In the animals presenting foul mouth and bloody diarrhea the chief interest centers in the lower bowel and rectum which exhibit an intense hemorrhagic appearance. With those animals dying rapidly from convulsions the only visible abnormality of the alimentary tract is the presence in the duodenum of one or more large ulcers.

In their summary the authors state that "from the facts enumerated the conclusion seems tenable that the abnormal state may be referred to a deficiency of some essential dietary constituent or constituents, presumably belonging to the group of hitherto unrecognized but essential components of an adequate diet."

Somewhat later Wheeler, Goldberger and Blackstock (2) called "attention to the striking similarity and probable identity of Chittenden and Underhill's pellagra-like syndrome in dogs and the condition known to American veterinarians as 'black tongue'." There is "the possibility, if not the probability, that black tongue in dogs may prove to be the analogue of pellagra in man." "Black tongue seems to have a geographic distribution in the United States singularly like that of pellagra. Seasonally it is reported to occur more frequently in summer and autumn and to affect our dogs less than those of higher grade. There is some evidence that it may occur more than once in the same animal."

In 1920 Cary (3), upon the basis of Chittenden and Underhill's report, classified black tongue among the deficiency diseases.

Quite recently Goldberger (4) and his associates have stated "that the substances that have been found to possess black tongue-preventive potency have, when tried, been found efficient preventives of the human disease and that those that had failed in pellagra, or were of low pellagra-preventive potency, when tried in black tongue have failed, or were feeble, as preventives of the canine disease."

The importance of the experimental production of this pathological condition for human nutrition was so obvious as to warrant further investigation. Since January 1918 an effort has been made to determine the cause or causes leading to this condition. The results obtained form the basis of this report. It should be emphasized that no attempt is made here to correlate this canine disease with human pellagra or even to endeavor to establish its identity with so-called black tongue. Instead it is our intent to regard the experimental condition as an entity and from this viewpoint to develop our argument to its logical conclusion.

EXPERIMENTAL. *General methods.* So far as they are applicable in the present investigation, the methods employed by Chittenden and Underhill have been adopted. Attention should be called to the fact that the experimental dogs were full-grown but young, vigorous animals of both sexes.

An endeavor was made to obtain dogs approximately two to three years old, free from all skin troubles or other visible defects. They were maintained in large, airy pens, with cement bottom, a portion of which was covered with either sawdust or shavings. Each animal occupied a separate pen, and an endeavor was made to maintain a fairly uniform temperature.

Upon their arrival at the laboratory the dogs were thoroughly washed with soap and water containing a small quantity of kerosene. An attempt was made to keep them free from fleas by use of proper insecticides. Strict cleanliness was observed throughout. In the selection, care and maintenance of the dogs we are indebted to Dr. G. Leroy Cheney, an experienced veterinarian.

All food was of good quality and carefully prepared. Nitrogen estimations were made on the food; calories were calculated. Food was given once a day; water as desired. Particular attention was paid to the preparation of the butter fat. The butter was melted in an electric oven and the fluid fat siphoned off. After filtration the butter fat was kept in a suitable refrigerator. The peas fed were ordinary dried peas well soaked in water and thoroughly cooked. They were allowed to soak in sufficient water so that after boiling the mixture was a semi-solid pasty mass. The length of boiling was usually about one hour. Peas were cooked for each meal only so that stale or sour food could play no rôle in the production of the diseased condition under discussion. It should be pointed out that when cottonseed oil is mentioned, the refined practically colorless product is meant.

Experiment 1. Repetition of Chittenden and Underhill's experiment. In initiating this investigation an attempt was made to duplicate the results of Chittenden and Underhill. For this purpose four dogs were placed upon a diet similar in every respect to that employed by these investigators. The ration follows:

Composition of food, expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Dried peas	3.57	0.15
Cracker meal	8.00	0.15
Cottonseed oil	5.00	
Total		0.30

This mixture was designed to furnish 0.3 gram nitrogen and 87 calories per kilo body weight, of which one-half of the total nitrogen was in the form of peas. The peas were cooked until soft.

At autopsy in all four animals the typical condition of the mouth was present. In three of the dogs congested areas in the intestine were noted

but ulcers were not found. The tissues were pale. From time to time blood examination had been made disclosing a red cell count approximating 5 to 7 millions per cubic millimeter, indicating that anemia did not play a significant rôle in the production of the pathological condition.

Conclusion. The experiment of Chittenden and Underhill was corroborated although the post-mortem findings were not so far advanced in the present series as in the former. One point of considerable diagnostic importance was established. When animals first show signs of loss of appetite, inspection of the mouth will reveal an inflamed condition of the gums as well as the inside of the lips. It is particularly prominent as a red line extending around the teeth somewhat analogous to the "blue line" of lead poisoning. Further experience has demonstrated this symptom of abnormality to be very characteristic and, together with loss of appetite, it constitutes the first conspicuous symptom of abnormality.

TABLE 1

Results of experiment 1

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
1	M	6.0	1.8	70	Foul mouth. Bloody diarrhea
2	F	9.8	2.7	79	Foul mouth. Bloody diarrhea. Also skin rash. Sore eyes. Birth to 2 dead pups
3	M	8.3	4.2	91	Foul mouth. Bloody diarrhea. Sore eyes
4	F	6.0	1.4	44	Foul mouth. Bloody diarrhea

THE PATHOLOGY OF THE DISEASE. In order to determine more exactly the pathology of the disease Dr. R. A. Lambert performed the autopsies on a large number of animals and studied the tissues microscopically.

Two typical protocols submitted by him are herewith appended as illustrative of the condition. The post-mortem examinations were made shortly after death.

Dog A_1. The body is very well nourished. The subcutaneous fat is quite abundant and normal looking. Over the back it forms a layer practically 1 cm. in thickness. The accompanying colored drawing (see fig. 1) shows very well the condition of the mouth as noted on external examination. The buccal mucosa in general is quite red, even hemorrhagic in patches, and irregularly covered with a fibrous purulent exudate. This change is much more marked laterally around the angles of the mouth than anteriorly. The gums are somewhat reddened but much less affected than the buccal mucosa. The inferior surface of the tongue is covered by a thick sheet of yellow exudate.

Heart—negative.

Lungs—negative.

Abdominal viscera. There is nothing noteworthy in any of these organs except for possible acute congestion of the liver.

Fig. 1. Drawing of mouth of dog with experimental disease

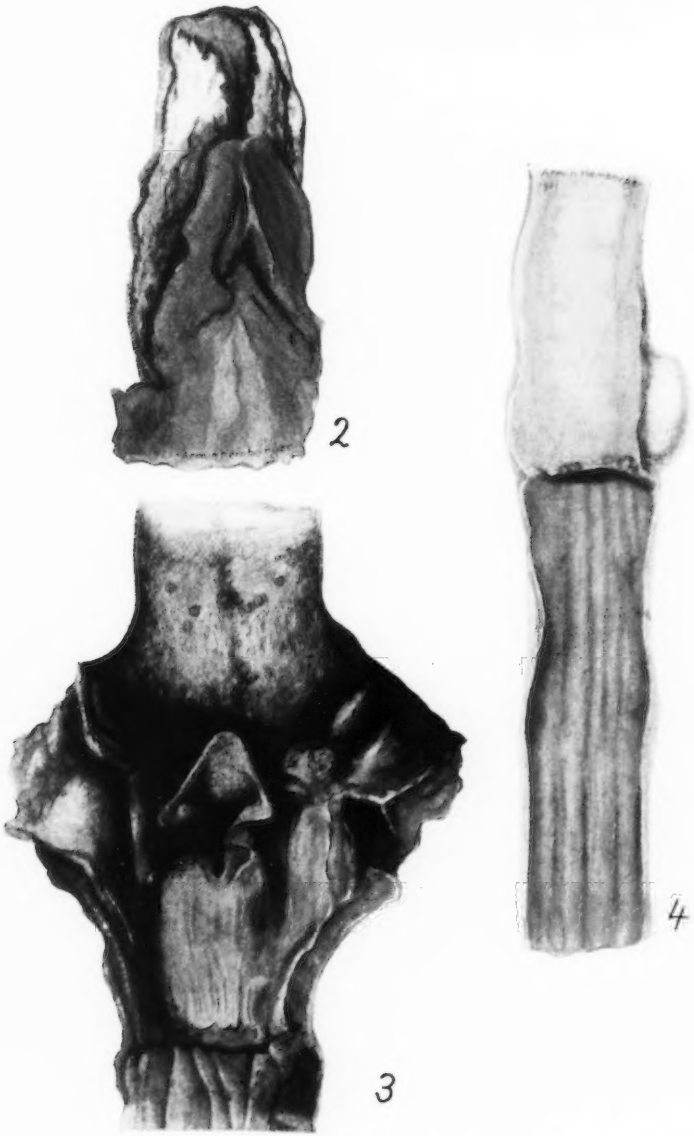

Fig. 2. Lesions of the tongue
Fig. 3. Lesions of the pharynx
Fig. 4. Lesions of the ileo-cecal junctions

Alimentary tract. The changes found in the tongue, pharynx and esophagus are well shown in the accompanying colored drawings (figs. 2 and 3). The anterior two-thirds of the superior surface of the tongue is clean and smooth, but the inferior surface, as noted above, is covered by a thick, yellow, membranous exudate which on being peeled off shows no hemorrhagic surface. The base of the tongue, the entire pharynx, and the upper two-thirds of the esophagus show a similar inflammatory reaction with a patchy fibrinous exudate over the entire surface. There is no distinct ulceration of the mucosa, however. The vessels are everywhere deeply injected and in places there are apparent hemorrhages. The trachea is practically unaffected, the inflammatory reaction stopping short at the epiglottis. The lymph nodes in the submaxillary regions are greatly swollen and deep red on section. The greater portion of the mucosa of the stomach is distinctly reddened but there is no exudate over this surface or erosions or hemorrhages. The mucosa of the entire small intestine is more or less injected. In the duodenum and jejunum there are a dozen or more swollen Peyer's patches which, with their depressed centers, simulate ulcers, but the mucosa is apparently intact throughout. In the lower portion of the ileum the vascular injection is quite pronounced and in the colon the surface presents an almost uniform red color with dark red lines corresponding to the crests of the longitudinal folds (fig. 4). The mucosa, as in the small intestine, is apparently intact throughout.

Bones. The long bones of both fore and hind legs, and the thighs were carefully examined for possible periosteal hemorrhages, but none were found. The ribs likewise show no gross pathological change.

Bone marrow. The bone marrow is deep red in color but not hemorrhagic.

Diagnosis. Acute diffuse stomatitis, pharyngitis, esophagitis, and ileocolitis.

Dog A_2. The body is poorly nourished but not markedly emaciated. There is still a small amount of subcutaneous fat. Externally the mouth shows practically the same picture as dog A_1.

Heart—negative.

Lungs—negative.

Abdominal organs. The liver shows the usual dark red color. The spleen, kidneys and adrenals are likewise negative.

Alimentary tract. The buccal cavity resembles that of dog A_1. The stomach is little altered. In the duodenum a few slightly swollen lymph follicles are seen and there is slight hyperemia. The congestion is more marked in the jejunum and ileum, becoming quite pronounced in the lower end of the latter, where small hemorrhagic foci stud the surface. The entire colon is quite hemorrhagic with a considerable quantity of mucus in the lumen.

Bones. Examination of the ribs and long bones of the extremities shows no periosteal hemorrhages.

Lymph nodes. The lymph nodes in the submaxillary region are quite swollen as in the previous cases.

Diagnosis. Acute fibrinopurulent stomatitis, pharyngitis, and ileocolitis.

In his report Doctor Lambert states that

in general the microscopic examination of the organs confirms the gross findings. From a review of the whole material I think certain points are fairly clear:

The lesions in the mouth and pharynx in the well-developed cases are quite characteristic. The buccal side of the lips, particularly near the angles of the mouth, the inferior surface of the tongue, pharynx, and the epiglottis are the site of predilection of the lesions. In a certain proportion of the cases the upper portion

of the esophagus was affected and in a still smaller number there was laryngeal involvement. It is rather surprising I think that an aspiration bronchopneumonia is not a more frequent complication. Microscopically the lesions of the mouth and pharynx present some interesting features. For instance, one is struck by the superficial character of the lesions and slight cellular reaction in the mucosa. I have not studied carefully the sequence of changes, but I have obtained the impression that the first change is a sort of hyaline degeneration and swelling of the subepithelial connective tissue and this is followed immediately by a sloughing of the epithelial layer. After the epithelium is lost there is some multiplication of vessels with the formation of a narrow zone of granulation tissue, but as I have indicated above, the reaction both on the part of the wandering cell as well as the fixed connective tissue cells is surprisingly slight. In some cases I have evidence of the regeneration of the epithelium. It should be noted that the microscopic sections show that the ulceration is not as widespread as the gross appearance would suggest so that except in the advanced cases healing might be expected. On the other hand, in the extreme case where the loss of epithelial covering is very widespread and secondary bacterial infection well established, a return to normal even when the etiological factor was removed could hardly be looked for. Regarding the intestinal changes I cannot speak so definitely. In the early cases I thought the changes were quite marked, and that there were beginning ulcers in the small intestine. Microscopic section shows, however, that I was in error in the gross interpretation. Apparently in the dog the atrophy of the lymphoid tissue which occurs in the course of malnutrition results in the formation of small depressed foci which superficially resemble ulcers. This appearance has been noted in emaciated control animals. I have also seen in the controls hemorrhages in the crests of the folds of the large intestines which I had at first interpreted as significant in the experimental dogs. Microscopically I think there may be certain changes particularly of an atrophic nature in the intestine. The glands are reduced in size and many seem to have disappeared. I should like, however, to study a number of controls very carefully before expressing final opinion on this point.

The question as to cause of death in a good many of the dogs is not satisfactorily explained by the autopsy. At least the anatomical changes did not appear sufficiently extensive to cause death. I wonder if there is not a very serious metabolic disturbance in such animals.

WHAT IS THE CAUSE OF THE ABNORMAL CONDITION UNDER DISCUSSION? If one analyzes the diet fed by Chittenden and Underhill several possibilities are at once evident to account for the failure of their animals to remain in good health. In the first place, it is possible that the character of the protein of the diet may play a predominent rôle. Again, even though the composition of the protein may not in itself be responsible, quantitatively, the amino acid content may be unsatisfactory. The content of fat soluble vitamin A is very low and it is also possible that the water soluble vitamin B may be insufficient. Moreover, although the total ash content seems adequate, the possibility exists that the diet was deficient in calcium and phosphorus. Finally, the nitrogen content is very low for a naturally carnivorous animal.

In the following pages an attempt has been made to take into account these possible factors one at a time, and by this means to establish a simple adequate diet upon which a dog may be maintained in health and vigor.

Experiment 2. The influence of bone ash added to the C-U[1] *diet.* To test the influence of added bone ash, consisting principally of calcium and phosphorus, two dogs were fed upon the C-U diet (described in experiment 1) plus 2 grams of bone ash per day.

Male dogs, 16 and 17, each lived on this diet for 86 days when they were both accidentally killed. Dog 16, with an initial weight of 6.5 kilos, lost 2.7 kilos, and dog 17, with an initial weight of 6.9 kilos, lost 3.2 kilos body weight. These animals although killed accidentally, may be fairly considered in the present discussion since they were both suffering from foul mouth and bloody diarrhea and were on the verge of death. In addition they both had an annoying skin rash.

Conclusion. So far as the experiment proceeded these animals presented symptoms typical of experiment 1 except that the terminal stage, death, was lacking. It is therefore evident that the addition of the elements present in bone ash to the diet did not prevent the appearance of typical symptoms.

Experiment 3. The influence of meat replacing a portion of the peas in the C-U diet. In this series meat replaced peas to the extent of an equivalent of two-thirds of the nitrogen contained in the latter, thus making the nitrogen of the meat equivalent to one-third the total nitrogen of the diet. Six dogs were thus fed. The composition of the ration follows:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	2.70	0.10
Dried peas	1.19	0.05
Cracker meal	8.00	0.15
Cottonseed oil	5.00	
Total		0.30

The intake of total nitrogen and calories adopted for the C-U diet remained unchanged.

At autopsy, in general, the characteristic appearance of the intestine was noted. Now and then an animal was found, for example, dog 9, in which intestinal congestion or hemorrhage were lacking.

Conclusion. Meat, at least in the quantities fed, failed to supply the requirement essential for life in these experimental animals.

Experiment 4. The influence of both meat and bone ash supplementing the C-U diet. Before making a more radical change in the diet the effect of adding both meat and bone ash was tested upon four dogs. To the ration of experiment 3 were added 2 grams of bone ash daily.

[1] Abbreviation for Chittenden-Underhill diet.

The results of this series are given in table 3.

At autopsy in dog 34 there were found, in addition to the foul mouth, hemorrhagic areas at the pyloric end of the stomach, apparent superficial ulceration in the duodenum, both old and new spots. The jejunal mucosa was injected and exhibited diffuse and superficial ulceration. The descending colon contained dark pigmented areas and yellow pin point spots.

In dog 35 the intestinal mucosa was diffusely injected with patches of yellowish mucus easily removable. There were a few focal injected areas but no necrosis. In the lower colon were about one-half dozen healed superficial ulcers 0.5 to 1.0 cm. in diameter.

TABLE 2

Results of experiment 3

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
7	M	6.8	2.9	63	Bloody diarrhea. Skin rash
8	M	7.9	2.8	56	Bloody diarrhea. Skin rash
9	F	6.6	0.8	35	Bloody diarrhea
14	M	6.7	0.8	24	Bloody diarrhea. Foul mouth
48	F	8.5	3.8	89	Diarrhea. Foul mouth
49	F	7.6	2.8	69	Diarrhea. Foul mouth

TABLE 3

Results of experiment 4

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
34	M	8.4	1.5	39	Bloody diarrhea. Foul mouth
35	F	8.6	2.7	55	Bloody diarrhea. Foul mouth
54	M	8.1	3.4	88	Bloody diarrhea. Foul mouth
55	F	8.7	2.4	36	Bloody diarrhea. Foul mouth

In dog 54 beyond a small degree of congestion in the intestine the most noticeable feature was the paleness of the tissues and general emaciation.

Dog 55 contained a tape worm and showed a general congestion of the intestinal tract. The mouth was moderately foul.

Conclusion. Bone ash plus a considerable quantity of meat modifying the C-U diet did not protect the animals under discussion. Neither were the characteristic untoward symptoms modified.

Experiment 5. The influence of replacement of peas in the C-U diet by meat. To test the possible specific influence of meat, the peas in the C-U diet were entirely replaced by lean raw meat, the tests being carried out with two dogs.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.05	0.15
Cracker meal	8.00	0.15
Cottonseed oil	5.00	
Total		0.30

It will be noted that of the total nitrogen of the diet one-half was now in the form of meat. This ration furnished approximately 83 calories per kilo of body weight.

On autopsy dog 15 showed small diffuse hemorrhagic areas throughout the intestinal tract. Dog 18 had red injected areas in the duodenum and an old superficial ulcer; in the rectum and colon were found rare scarred areas and dark pigmented areas. The cornea of each eye was opaque with perforation in each eye. In the left eye the anterior chamber contained a slight amount of yellow pus and the iris was somewhat broken down. The chest contained a small abscess and the skin in general was the seat of an active rash.

Conclusion. Replacement of peas in the C-U diet by lean raw beef failed to maintain dogs and they died with the typical symptoms. It must be pointed out, however, that the average length of life was greater than when a smaller quantity of meat was fed along with peas.

Experiment 6. The influence of higher nitrogen intake. Chittenden and Underhill state that "the level of nitrogen intake of itself has little or nothing to do with the result obtained." Their experience was gained, however, upon animals to which larger quantities of peas had been fed and also in which part of the peas had been replaced by casein. It was deemed of importance to determine anew whether a similar result would be obtained when the nitrogen content of the C-U diet had been augmented in part by raw meat. Three dogs accordingly received the following ration:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.05	0.15
Dried peas	7.14	0.30
Cracker meal	8.00	0.15
Cottonseed oil	5.00	
Total		0.60

In this mixture the total nitrogen intake is double that of the C-U diet and the calorific intake is somewhat increased.

Autopsy revealed the usual abnormal appearances of the mouth and intestinal tract.

Conclusion. The statement of Chittenden and Underhill that "the level of nitrogen intake of itself has little or nothing to do with the result obtained" finds confirmation in the present experiment even when the additional nitrogen was in part supplied by meat. It must be noted, however, that the average length of life was considerably greater than when dogs were maintained on a lower plane of nitrogen intake.

TABLE 4

Results of experiment 5

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
15	M	7.8	1.9	63	Bloody diarrhea. Foul mouth
18	M	9.2	4.9	116	Bloody diarrhea. Skin rash

TABLE 5

Results of experiment 6

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
50	F	9.4	1.3	83	Bloody diarrhea. Foul mouth
52	F	10.4	1.8	114	Bloody diarrhea. Foul mouth
56	M	10.8	1.0	89	Bloody diarrhea. Foul mouth

Experiment 7. Replacement of a portion of the peas in the C-U diet by liver. Inasmuch as the liver is rich in vitamins essential for the maintenance of adult animals, in the present experiment two-thirds of the nitrogen of the peas were replaced by pig's liver, so that of the total nitrogen intake one-third was derived therefrom.

The composition of the diet fed follows:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Pig's liver	2.80	0.10
Dried peas	1.19	0.05
Cracker meal	8.00	0.15
Cottonseed oil	5.00	
Total		0.30

On autopsy dog 10 presented a normal appearance except for skin rash. Dogs 11 and 12 exhibited characteristic changes in the intestinal tract.

Discussion. Compared with the other two dogs, dog 10 showed marked differences both in the symptoms and length of life; and these peculiarities call for some consideration. Dog 10 died without any typical manifestations. Loss of appetite was the most significant feature to be observed. Nevertheless this animal ate more or less food up to the day of death. During the course of the experiment this dog gave birth to four pups, two of which she suckled for a period of more than one month. The marked differences peculiar to this dog are undoubtedly due to the preparation of the liver fed. In the beginning all three dogs were fed with pig's liver that had been air dried. The fresh liver was put through a grinder, spread in a thin layer on large porcelain plates and dried by being placed about two feet above large steam radiators. After the death of dogs 11 and 12 the

TABLE 6

Results of experiment 7

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
10	F	9.8	1.7	246	Skin rash only
11	F	8.7	1.4	67	Bloody diarrhea. Foul mouth
12	M	5.3	1.8	82	Bloody diarrhea. Foul mouth

TABLE 7

Results of experiment 8

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
32	M	10.4	3.5	284	Skin rash only. Used for other purposes
33	M	9.1	2.3	284	Skin rash only. Used for other purposes

feeding of dried liver was discontinued with dog 10 and in its place fresh liver, or liver preserved in a frozen state was fed. It seemed probable from this experience that heating to the degree indicated above had injured or destroyed some potent factor present in the fresh liver.

Conclusion. Pig's liver, not subjected to heat, when introduced in appreciable quantity into the C-U diet is capable of greatly prolonging the life of the experimental animal. When the same food material is dried at a relatively low temperature and fed under comparable conditions, it fails to maintain life for a period longer than may obtain with the original C-U diet.

Experiment 8. The substitution of liver for peas in the C-U diet. The apparent success with dog 10 in experiment 7 warranted a further trial of the influence of liver in the maintenance of life; consequently the diet was

so selected that liver (either fresh or kept in a frozen condition) replaced entirely the peas in the C-U diet. Such a modified ration was fed to two dogs.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Pig's liver	4.2	0.15
Cracker meal	8.0	0.15
Cottonseed oil	5.0	
Total		0.30

These dogs were kept upon this diet for a period of 284 days, an interval sufficiently long to demonstrate that this diet is adequate to maintain life over a period much more prolonged that is possible with the C-U diet. It is true that the dogs lost heavily in body weight and also suffered from an

TABLE 8

Results of experiment 9

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
4	F	9.8	4.9	104	Refused to eat. Diarrhea. Foul mouth
5	M	9.4	3.6	89	Bloody diarrhea. Skin rash. Foul mouth
6	F	11.2	3.8	108	Bloody diarrhea. Skin rash. Foul mouth

annoying skin rash. In spite of the last cited facts the animals were in excellent nutritive condition.

Liver contains both fat soluble A and water soluble B vitamins and the thought lies near that, one or the other, or both, of these vitamins may be responsible for the nutritive improvement of the C-U diet after the addition of either fresh or frozen liver. It seemed unlikely that the water soluble B vitamin is the responsible agent since both dried peas and fresh beef contain some of this vitamin and neither peas nor meat are able to maintain life under the selected experimental conditions. It therefore was important to determine whether the fat soluble vitamin A is the responsible agent. We shall return to this problem shortly.

Conclusion. Pig's liver, either fresh or kept in a frozen condition, when substituted for peas in the C-U diet makes the diet capable of maintaining life for prolonged periods.

Experiment 9. The influence of a small amount of butter upon the C-U diet. In an endeavor to determine the potent factor present in liver another source of fat soluble vitamin A was sought which would be free from

the water soluble B. Butter fat seemed to be the ideal substance for the purpose. Accordingly the latter especially prepared (see under General Methods, page 5) was substituted for a part of the cottonseed oil in the C-U diet, and the modified ration fed to three dogs.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Dried peas	3.57	0.15
Cracker meal	8.00	0.15
Cottonseed oil	3.30	
Butter fat	1.70	
Total		0.30

This experiment was also intended as a comparison with experiment 1 (original C-U diet).

Conclusion. Butter fat added to the C-U diet in place of one-third of the cottonseed oil failed to maintain life and to prevent the appearance of the typical symptoms. If butter fat is effective it must be potent only in doses greater than those given here. However, comparison with the results of experiment 1 leads to the conclusion that the average length of life is greater when butter fat is included in the diet.

Experiment 10. The influence of butter fat in an otherwise inadequate diet. In experiment 3 it was pointed out that when a portion of the peas of the C-U diet is replaced by meat little or no improvement is to be observed. In the present experiment the diet employed in experiment 3 was used except that butter fat entirely replaced the cottonseed oil. Two dogs were fed with the diet.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	2.70	0.10
Dried peas	1.19	0.05
Cracker meal	8.00	0.15
Butter fat	5.00	
Total		0.30

Dog 25 died suddenly with atypical symptoms. From the autopsy report it is quite probable that pneumonia caused death.

Dog 37, at the end of 206 days, was in good nutritive condition despite the skin rash and a considerable loss of body weight. With the object of attempting to alleviate the skin condition a salt mixture consisting of

NaCl 1.0 gram, calcium lactate 3.0 grams, $FeCl_3$ 5.0 mgm., and sodium iodide 2.0 mgm. was given daily. The diet thus modified was continued for 114 days longer during which time the dog gained 0.1 kilo in body weight. The skin improved considerably but the eyes continually contained pus in spite of local treatment. Eventually both eyes became cloudy and the animal was practically blind. Death occurred with atypical symptoms.

Autopsy of dog 37 indicated a pneumonia process. The intestinal tract, full of food, did not afford any indication of sufficient abnormality to account for death. The eyes were filled with pus and the right cornea was perforated.

Conclusion. It is quite apparent that butter fat added to the otherwise inadequate diet greatly prolongs life. Loss of body weight and skin rash were, however, not prevented. The influence of butter fat is quite evident when comparison is made of the length of life of animals in experiment 3 (with cottonseed oil) and those of the present experiment.

TABLE 9

Results of experiment 10

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
25	M	4.4	1.7	126	Skin rash only
37	M	8.7	2.2	206	Skin rash only

TABLE 10

Results of experiment 10a

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
41	F	7.0	0.4	146	No symptoms of any sort
42	M	6.8	0.1	205	No symptoms of any sort

Experiment 3	*Days of life*	*Present experiment (10)*	*Days of life*
Dog 7	63	Dog 25	126*
Dog 8	56	Dog 37	320*
Dog 9	35		
Dog 10	24		
Dog 48	89		
Dog 49	69		

*Both presumably died of pneumonia.

The experience gained with experiment 10 was so significant that further evidence was sought. Dogs 41 and 42 were fed a diet similar in every

respect to that of experiment 10 except that there were added 2 grams of bone ash instead of the salt mixture. The results are given in table 10 following.

Autopsy revealed nothing to account for death nor were the usual intestinal appearances present.

Conclusion. It is quite evident that the addition of butter fat to the inadequate diet, under the experimental conditions prevents the appearance of typical symptoms and greatly prolongs life. In these animals skin and eye troubles were not noteworthy. It is quite possible that the addition of bone ash afforded some protection against the skin rash.

To test out the latter point two more dogs were fed on the above diet from which bone ash was omitted. The results may be seen in table 11.

TABLE 11

Results of experiment 10b

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
51	F	9.7	1.2	184	No symptoms except loss of hair
53	M	11.8	0.2	136	No symptoms except loss of hair

TABLE 12

Results of experiment 11

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
46	M	8.9	0.6	204	No symptoms. Experiment stopped
47	M	12.3	3.0	141	No symptoms. Experiment stopped

These animals were in excellent nutritive condition except that after a time a moderate degree of skin rash appeared which eventually cleared up. Coincident with the skin rash about one-half of the hair of the body fell out and was not restored.

Conclusion. The conclusion of experiment 10 with respect to the influence of butter fat was corroborated. Bone ash did not completely protect against skin rash and a new symptom—irreparable loss of hair—appeared.

Experiment 11. The replacement of peas by meat and of cottonseed oil by butter in the C-U diet. The suggestive results in experiment 10 with butter fat led to the test of the influence of meat entirely replacing peas in this dietary. Two dogs were fed this modified diet.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.05	0.15
Cracker meal	8.00	0.15
Butter fat	5.00	
Bone ash	2.00	
Total		0.30

Discussion. From the tabulated results it is quite apparent that the diet in experiment 11 is capable of maintaining dogs over a prolonged period. Dog 46 lost 0.6 kilo and no significant symptoms were observed. The other dog, in the course of time, suffered loss of hair and also skin rash which eventually almost disappeared. It is, of course, quite possible that the large loss of body weight in the one animal was associated with the skin rash and the loss of hair.

TABLE 13

Results of experiment 12

DOG	SEX	INITIAL WEIGHT	GAIN IN WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
46	M	8.3	2.8	102	No symptoms. Skin rash gone
47	M	9.3	0.4	102	No symptoms. Skin rash gone

Conclusion. Butter fat undoubtedly supplies a potent factor in prolonging life of dogs on the otherwise inadequate diet. It would also appear that the addition of meat alone is without significant influence in this respect.

Experiment 12. The influence of larger quantities of meat. Although the larger quantity of meat in experiment 11 seemed to be without especial significance it was of interest to determine whether decidedly greater quantities would exert any influence. Accordingly the following diet was fed to dogs 46 and 47 previously employed in experiment 11.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	16.2	0.60
Cracker meal	8.0	0.15
Butter fat	5.0	
Total		0.75

Conclusion. More liberal meat intake maintained the dogs in good nutritive condition; both gained weight and the previous skin rash entirely disappeared. It is, therefore, apparent that a higher meat intake was beneficial although not absolutely essential for life.

Experiment 13. The effect of a moderate intake of meat and the influence of butter fat. This experiment was designed to determine the influence of a moderate quantity of meat as opposed to the large amount fed in experiment 12. For this purpose two dogs were given the following diet:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	8.1	0.30
Cracker meal	8.0	0.15
Butter fat	5.0	
Total		0.45

Conclusion. A moderate quantity of meat maintained these dogs in good nutritive condition so that both gained in weight. One of the animals suffered from a slight skin rash which persisted throughout the experiment. Again, the smaller gain in weight occurred in the animal with the skin rash, which points to a relationship between lack of gain and the skin condition.

Experiment 13a. The influence of butter fat. It is quite apparent that the diet detailed above for experiment 13 is sufficient to maintain dogs in good nutrition. In order to demonstrate the rôle of butter fat in this connection these two dogs were kept on the same diet except that butter fat was replaced by an equivalent quantity of cottonseed oil. The results may be seen in table 15.

Discussion. On autopsy the striking feature with dog 39 was emaciation and the generally anemic appearance of the tissues. The intestinal tract contained areas of congestion and a few hemorrhagic areas.

With dog 40 the general nutritive condition was good. The intestinal canal showed typical hemorrhagic areas and the mouth was red and congested.

Dog 39 ate well for the first 45 days and during this period lost 1.1 kilos: during the remaining 46 days a smaller quantity of food was eaten daily which accounts for the greater loss in weight. It is of considerable interest that generally when an animal eats well of a diet, defective as this one was, in the end more striking symptoms are evoked and death intervenes rapidly. On the contrary, when a dog eats only a minimum of food less obvious symptoms arise and life is maintained for a relatively long period. It would appear that the failure to eat is a sort of protective mechanism—

perhaps a conservation of vital substances, which apparently are used up much faster when a greater quantity of food is ingested. It may also be true that through tissue disintegration in starvation certain essentials are "liberated" from the tissues. Rickets, for example, tends to heal during loss of weight.

Conclusion. The elimination of butter fat from the adequate diet, containing a moderate amount of meat, rendered the diet incapable of maintaining life although death was considerably delayed indicating either that meat affords a certain degree of protection or that the previous feeding with butter fat had conferred upon the organism a greater resistance. This experiment, again, indirectly demonstrated the rôle of butter fat in protection against the diseased condition under consideration.

TABLE 14

Results of experiment 13

DOG	SEX	INITIAL WEIGHT	GAIN IN WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
39	M	10.0	1.6	182	No symptoms of any kind
40	F	7.7	0.9	169	Slight skin rash

TABLE 15

Results of experiment 13a

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
39	M	11.6	5.6	91	Bad skin rash. General malaise. Poor appetite. Anemic
40	F	8.6	0.4	100	Skin rash. Diarrhea. Foul mouth

Experiment 14. The influence of liver and butter in the C-U diet. It will be recalled that in experiment 8, dogs 32 and 33 were maintained for 284 days on the C-U diet when the peas were replaced by liver. The animals lost weight and suffered from skin rash but otherwise were in excellent condition. These dogs were then placed upon the following ration:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Pig's liver	4.2	0.15
Cracker meal	8.0	0.15
Butter fat	5.0	
Total		0.30

These animals lived on this diet for a period of 234 days when the experiment was stopped. The skin rash disappeared and both dogs were in splendid condition. Dog 32 gained 4.9 kilos and dog 33 0.5 kilo during this period.

Conclusion. The addition of butter fat to the diet of experiment 8, (although the latter itself suffices for the maintenance of life), is of marked benefit to the general nutrition of the dog. It will not only maintain life under these conditions but the animal gains in weight and so far as one may judge is in a perfect state of nutrition.

Repetition of experiment 14. In order to confirm these conclusions an experiment was carried out on four dogs with the same diet (experiment 14) except that with two dogs 2 grams of bone ash were given per day, and to the other two the same amount of bone ash plus 1 gram of sodium chloride. The addition of bone ash and common salt would seem to give

TABLE 16

Results of experiment 14

DOG	SEX	INITIAL WEIGHT	LOSS IN WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
29	M	6.2	1.5	219	Pus in eyes. Skin rash. Experiment stopped
36	M	9.6	1.6	207	Great loss of hair. Skin rash. Experiment stopped
30	F	3.7	1.2	93	Skin rash. Death without symptoms
38	F	7.0	1.5	202	No symptoms. Experiment stopped

an almost perfect completeness to the diet so far as the now known foodstuffs and food accessories are concerned.

Dogs 29 and 36 received bone ash: dogs 30 and 38 received bone ash plus common salt.

Of these four animals three lived for extended periods, the only outstanding features being a skin rash, loss of weight, loss of hair and at times pus in the eyes. They all lived for a sufficiently long period to demonstrate the success of maintenance and then were used for other purposes. Dog 30, however, lived only 93 days and died without symptoms. At post-mortem examination no definite abnormality could be detected.

Conclusion. So far as the skin rash and loss of weight are concerned this experiment does not corroborate that of the previous experiment (experiment 14). It does, however, confirm the conclusion that this diet can maintain life for prolonged periods.

Continuation of experiment 14. Dog 38 of the above experiment was placed on the diet above except that peas were added and somewhat increased the intake of total nitrogen.

The composition of the food follows:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Pig's liver	4.2	0.15
Dried peas	3.6	0.15
Cracker meal	8.0	0.15
Butter fat	5.0	
Total		0.45

Bone ash ..... 2 grams per day
Sodium chloride ..... 1 gram per day

Upon this diet dog 38 lived for 304 days, gained 1.5 kilos and no symptoms of any kind were observed. The experiment was then stopped.

Conclusion. The addition of nitrogen in the form of peas caused this dog, which had steadily lost weight, to regain its original weight. It would seem that the higher nitrogen intake associated with these experiments is correlated with increase in weight, even though the augmented nitrogen was in the form of peas.

Further evidence of the beneficial influence of a high nitrogen intake. Two dogs were placed upon a diet containing meat, liver, cracker meal, butter fat and a salt mixture. The meat and liver were fed in such quantities that the nitrogen content from the two protein sources was equal. The details of the diet follow:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	8.1	0.30
Pig's liver	8.4	0.30
Cracker meal	8.0	0.15
Butter fat	5.0	
Total		0.75

Salt mixture per day:
Sodium chloride ..... 1 gram
Calcium lactate ..... 3 grams
Ferric chloride ..... 5 milligrams
Sodium iodide ..... 2 milligrams

The dogs lived on this diet for 182 days without symptoms when the experiment was discontinued.

Conclusion. Apparently the gain in body weight under the present experimental conditions is directly associated with increased nitrogen intake. It is also noteworthy that when animals gain in this way skin affections are lacking.

TABLE 17

Results of experiment 14

DOG	SEX	INITIAL WEIGHT	GAIN IN WEIGHT	PERIOD ON DIET	REMARKS
		kilos	*kilos*	*days*	
43	F	7.0	1.6	182	No symptoms
44	M	7.8	2.0	182	No symptoms

TABLE 18

Results of experiment 15

DOG	SEX	INITIAL WEIGHT	LOSS IN WEIGHT	LENGTH OF LIFE	PERIOD WITHOUT YEAST	PERIOD WITH YEAST	BLOOD COUNT		REMARKS
							White	Red	
							In thousands per cmm.	In millions per cmm.	
		kilos	*kilos*	*days*	*days*	*days*			
A_1	F	11.3	1.5	49	33	16†	22*	7.2	Appetite regained. Diarrhea. Foul mouth
							16	8.7	
A_2	F	7.4	0.9	56	30	26	6	8.1	Appetite regained. Diarrhea. Foul mouth
							8	8.0	
A_3	F	7.6	0.2	25	25	0	13	5.4	Appetite maintained, hence no yeast. Diarrhea. Foul mouth
							9	7.0	
A_4	M	10.0	3.0	65	33	32	12	8.6	Appetite regained. Diarrhea. Foul mouth
							11	8.4	
A_6	M	13.8	4.2	49	25	24	7	7.3	Appetite regained. Death in convulsions
							11	6.4	
A_7	M	7.0	1.3	34	34	0	10	5.8	Appetite maintained, hence no yeast. Diarrhea. Foul mouth
							29	6.2	

* In these brackets the first figures are those obtained at the beginning of the experiment; the second those figures yielded near the conclusion.

† In this experiment one yeast cake per day.

Experiment 15. The influence of yeast. At this stage in the progress of our investigation the significance of yeast for the stimulation of appetite became established and the importance of this phase of nutrition to our own problem was obvious. Accordingly we wish to report a series of experiments planned with the view of testing the influence of yeast in the alleviation of the syndrome of symptoms characteristic of the disease forming the subject of this report.

The plan followed was to place dogs upon diets considered inadequate from our experience and at the first hint of falling off of desire for food to supplement the diet by the addition of compressed yeast. In the first series of experiments the composition of the food was as follows:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.3	0.15
Cracker meal	8.8	0.15
Cottonseed oil	5.0	
Salt mixture*	4.9	
Compressed yeast—part of the time		
Total		0.30

* Hereafter when reference is made to a salt mixture the following is meant:

Sodium chloride	10 grams
Calcium lactate	4 grams
Magnesium citrate	4 grams
Ferric citrate	1 gram
Lugol's solution	Few drops

The mixture was fed in quantities equivalent to 2.0 per cent of the total food intake.

The results outlined in table 18 are clear cut and leave no doubt relative to the influence of the added yeast. As soon as the animal indicated loss of appetite yeast was given. In every such case the appetite was almost immediately stimulated and all food was consumed until a day or two before death. In spite of the yeast, however, typical symptoms appeared and death resulted. In various animals suspicion of an anemic condition arose owing to the marked paleness of some of the tissues, the muscles in particular. The blood count of these animals as given in table 18 would not bear out such a conception.

Conclusion. It is evident that yeast, in the quantity here given, which is presumably adequate to cover the needs of the dog for water soluble vitamin B, fails, under the experimental conditions, to protect the animal

against the appearance of the symptoms characteristic of the disease, and it likewise fails to prolong life.

Experiment 16. The influence of yeast. The present experiment differed in two particulars from the preceding experiment, namely, *a*, casein[2]

TABLE 19

Results of experiment 16

DOG	SEX	INITIAL WEIGHT	LOSS IN WEIGHT	LENGTH OF LIFE	PERIOD WITHOUT YEAST	PERIOD WITH YEAST	BLOOD COUNT		REMARKS
							White	Red	
							In thousands per cmm.	In millions per cmm.	
		kilos	*kilos*	*days*	*days*	*days*			
A_8	F	8.2	3.2	47	16	31†	11*	8.0	Appetite regained. Diarrhea.
							3	7.0	Inflamed mouth
A_9	F	8.7	0.6	27	21	6†	10	8.0	Appetite not regained. Bloody
							10	9.1	diarrhea. Sore mouth
A_{10}	F	8.7	5.2	114	16	37†	10	8.7	Ate very little food. No char-
						61‡	4	5.9	acteristic symptoms
A_{11}	M	10.9	2.5	89	18	37†	11	8.4	Appetite regained. Diarrhea.
						34‡	8	6.1	Slimy mouth
A_{12}	M	12.0	3.1	81	17	47†	5	12.5	Appetite never regained.
						17‡	32	2.1	Symptoms not character-
									istic
A_{13}	M	12.8	2.6	38	22	16†	13	11.7	Appetite regained. Bloody
							14	7.8	diarrhea. Foul mouth

* Beginning and end of experiment.

† 1 yeast cake per day.

‡ 2 yeast cakes per day.

replaced meat in the dietary, and *b*, in certain instances larger quantities of yeast were fed.

[2] The casein was obtained from Merck and was three times extracted with boiling alcohol to free from fat. This casein has been shown to be free from water soluble vitamin B and presumably the alcohol extraction also freed it from fat soluble A.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Casein	2.1	0.15
Cracker meal	8.8	0.15
Cottonseed oil	5.0	
Salt mixture		
Yeast		
Total		0.30+

It is obvious that, as in experiment 15, yeast failed to protect the animals against the disease. On the other hand, in those dogs receiving two yeast cakes per day life was prolonged. In dog A_{13} a definite anemia was present

TABLE 20

Results of experiment 17

DOG	SEX	INITIAL WEIGHT	CHANGE IN WEIGHT	LENGTH OF LIFE	PERIOD WITHOUT YEAST	PERIOD WITH YEAST	REMARKS
		kilos	*kilos*	*days*	*days*	*days*	
C_{14}	F	8.9	0.3 loss	244	161	83*	No symptoms except occasional loss of appetite during warm weather. Skin maintained in excellent condition. Killed for autopsy
C_{15}	F	8.6	1.9 gain	244	231	13†	No symptoms except occasional loss of appetite during warm weather. Skin maintained in excellent condition. Killed for autopsy
C_{16}	M	8.9	1.0 gain	244	237	7*	No symptoms except occasional loss of appetite during warm weather. Skin maintained in excellent condition. Killed for autopsy

* 1 yeast cake per day.
† 2 yeast cakes per day.

accompanied by a great increase in the white cells, which is due, perhaps, to a pneumonic process as determined at autopsy.

Conclusion. Although yeast may prolong life it does not protect against the disease nor does it prevent death.

Experiment 17. The demonstration of an adequate diet for the dog. Our experience as detailed in the present report has led to the view that some factor present in liver and in butter fat is essential for continued good nutrition in the dog. We adopted this viewpoint as a working hypothesis and the experiment now to be described was planned to demonstrate the

correctness of this position. Three dogs were fed upon the diet detailed below:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Butter fat	5.0	
Salt mixture (2 per cent of total food)		
Yeast		
Total		0.30+

Discussion. In this experiment yeast was added to the diet at intervals. It has been sufficiently demonstrated in previous experiments in this report that yeast will not protect against the onset of the disease symptoms. On the other hand, it is also clear that yeast will stimulate a sluggish appetite. In recognition of this fact this adjuvant to good nutrition has been variously employed in this experiment. After dog C_{14} had been on the diet for 155 days a spell of hot July weather was encountered and the dog did not eat all the food. To stimulate the appetite one yeast cake was given daily for a period of 83 days during which interval the appetite was gradually regained but its return was much delayed.

Dog C_{15} consumed the entire food for a period of 137 days when this animal also began to show evidences of failure of appetite, presumably influenced by the same interval of warm weather. To this dog two yeast cakes per day were given for 13 days. The appetite responded and yeast administration was stopped. No more yeast was given throughout the remainder of the experiment which continued for 94 days beyond this period.

The appetite of dog C_{16} did not fail until 164 days had passed. The administration of one yeast cake daily for seven days was sufficient to cause this animal to resume normal consumption of food.

These three dogs, unlike most of those previously reported, even though on a low level of nitrogen intake were maintained in excellent condition. Dog C_{14} suffered a slight loss of weight, 0.3 kilo, too small to be of much significance. The other two animals gained in weight. These dogs were autopsied and Doctor Lambert reported negative findings throughout.

Conclusion. A diet consisting of meat, cracker meal, butter fat, and a salt mixture is adequate to maintain dogs in health and excellent nutritive condition for prolonged periods. Although not essential, it may probably be accepted that yeast added to the above diet is beneficial.

It may further be accepted that this diet protects against the incidence of the disease under discussion.

SUMMARY OF CONCLUSIONS

The experimental production of the diseased condition in dogs reported by Chittenden and Underhill has been confirmed.

The pathology of the disease has been studied more closely disclosing that the structural changes are localized throughout the alimentary canal but are particularly prominent in the buccal cavity. The disease is not scorbutic in character.

The incidence of the disease is not caused by the lack of inorganic salts in the diet.

Meat in the diet will not prevent the appearance of the symptoms although they may be considerably delayed, especially with relatively large quantities of meat.

High nitrogen intake, whether in the form of peas or meat, will not prevent the incidence of the disease although the symptoms may be somewhat delayed. It would appear, however, that increase of, or maintenance of body weight is more easily attained with a high nitrogen level than when smaller quantities of nitrogen are ingested.

Yeast, at least as supplied in the form of commercial compressed yeast, and in the quantities fed in these experiments, cannot be regarded as containing the protective factor. It may prolong life, but eventually the animal succumbs with typical symptoms.

Fresh pig's liver prevents the incidence of the disease. Similar liver dried at a relatively low temperature loses a part, at least, of this potency.

Butter fat contains the factor effective in the prevention of the disease.

PART II. THE ALLEVIATION AND CURE OF THE DISEASE. *The curative properties of butter fat.* It has been established that butter fat in the diet of the dog, under experimental conditions detailed earlier, will protect the animal against the appearance of the symptoms characteristic of the disease under discussion, and thus serve to maintain life for prolonged periods.

In order to demonstrate even more clearly the rôle of butter fat in this connection we have carried through a series of experiments in which the disease has been induced, and have attempted to cure it by the administration of butter fat. In this connection it must be pointed out that the cure of the disease once initiated is much more difficult than is its mere prevention. So many factors enter into an endeavor of this sort that many failures must naturally be expected. On the other hand a single demonstration of a curative action will greatly outweigh many failures. Obviously the chief feature which determines the success of such an experiment is the sufficiently early recognition of definite symptoms. Success can only be anticipated when the stage of the disease has not progressed

beyond possible hope of readjustment of metabolic processes or has proceeded to the point where the irremediable pathological structural changes have begun.

The present series of experiments may be arranged into three groups, as follows:

Group I. Experiments in which dogs were cured of the disease and were then maintained for extended periods of time.

Group II. Experiments in which dogs were cured of the disease but in which butter fat failed to maintain them for long periods of time.

Group III. Experiments in which butter fat failed to cure.

Experiment 18. Experiments on the curative action of butter fat. General plan. Dogs were fed at first appropriate quantities of the C-U diet plus yeast in certain instances and also plus the salt mixture. They were kept upon this diet until the first evidence of abnormality appeared. The criterion adopted was the development of redness of the gums and inside of the lips which rapidly follows loss of appetite. Our experience has been sufficient to warrant us employing these symptoms as evidence of the initiation of the abnormal state. At this point the cotton seed oil of the diet was replaced by equivalent quantities of butter fat, and at times yeast was added in greater quantity in an endeavor to stimulate a persistently poor appetite.

In all the experiments dealing with curative properties of different substances, if the animal refused food, the curative substance plus a small portion of meat, or at times also yeast, was force fed. Such a condition of affairs usually prevailed for a few days only, for the dog either regained its appetite or else died.

Control experiments. In this investigation the environment and general experimental conditions have been so uniform that control experiments hardly seem pertinent. Nevertheless in order to determine the possibility of continued maintenance under the experimental conditions one dog was placed with the experimental animals on October 15, 1921. It received no definite diet but was fed on scraps, bread, meat, dog biscuit, etc., in fact, any kind of food that was convenient. It remained in excellent condition until February 1, 1923 when it was found dead. During this period it had gained one kilo in bodyweight. Autopsy revealed no cause of death.

A second dog under like conditions served as control from February 6, 1923 until June 16, 1927, when the experiment was concluded. Its nutritive condition was excellent throughout and it had gained 4.9 kilos in body weight.

From these controls it is quite apparent that occasionally a dog will die from causes other than dietary restrictions. On the other hand, the second dog illustrates the feasibility of maintaining dogs in confinement without abnormal symptoms over long periods of time.

TABLE 21

Results of experiment 18—group I

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF BODY WEIGHT	PERIOD ON BUTTER FAT	GAIN IN WEIGHT	REMARKS
		kilos	*days*	*kilos*	*days*	*kilos*	
D_{17}	M	7.1	76*	1.8	171*	2.6	Died without symptoms. Pathological report—typical patchy bronchopneumonia. Body in excellent condition
D_{18}	M	14.7	25†	2.9	240†	3.2	Vomiting, diarrhea, inflamed mouth. Pathological report—characteristic features of the disease. Body in excellent condition
D_{21}	M	10.3	37‡	1.3	151†	1.6	Diarrhea. Inflamed mouth. Pathological report—typical mouth lesions, focal areas of bronchopneumonia. Body in excellent condition
D_{26}	M	13.0	40†	0.9	140†	2.6	Diarrhea. Inflamed mouth. Excellent condition. No autopsy
D_{33}	M	10.2	30‡	0.9	1689†	6.3	*Still living in excellent condition. Was maintained on this diet containing butter from Nov. 21, 1922 until June 16, 1927*
D_{42}	M	12.2	58§	0.9	111¶	2.4 loss	No symptoms but dog did not thrive as well as others. Used for other purposes
D_{43}	M	10.6	20‖	1.3	980¶	5.8	No symptoms throughout. Died of pneumonia—edema of lungs
D_{50}	M	10.6	28‖	0.6	162 ⁎	0.3	Typical symptoms before death
D_{51}	F	7.0	21‡	0.1	319†	0.0	Died with atypical symptoms. Autopsy findings were negative
D_{52}	F	12.5	18¶	1.6	149 ⁎	6.9	Dog in excellent condition throughout, then diarrhea, inflamed mouth. At autopsy alimentary tract without lesions
D_{58}	M	12.0	11‡	0.3	126△	3.1 loss	Dog in poor condition throughout. Entire behavior and death atypical. At autopsy no lesions

* Received 1 yeast cake per day at intervals. At terminal stages 2 yeast cakes were given.

† Received 1 and 2 yeast cakes per day at intervals.

‡ Received no yeast.

§ Received 2 yeast cakes daily for last 17 days on C-U diet.

¶ Received 2 yeast cakes daily.

‖ Received 2 yeast cakes daily for last 6 days on C-U diet.

⁎ Received 2 yeast cakes at intervals.

△ Received 1 or 2 yeast cakes at intervals.

TABLE 21—*Concluded*

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF BODY WEIGHT	PERIOD ON BUTTER FAT	GAIN IN WEIGHT	REMARKS
		kilos	*days*	*kilos*	*days*	*kilos*	
D_{61}	F	6.2	13‡	0.2	265†	0.6 loss	Dog in excellent condition. No symptoms. Used for other purposes
D_{65}	F	8.6	31 ○	0.2	470 □	0.0	No symptoms except loss of hair. Used for other purposes
D_{66}	F	8.7	23 ▲	0.5	705 △	1.1	No symptoms throughout. Used for other purposes
D_{70}	F	12.2	66 △	2.7	186 △	0.6 loss	Sudden death with no symptoms. Autopsy—edema of lungs
D_{71}	F	9.0	50 ●	0.2■	243 △	5.4	No symptoms except loss of hair for a period; hair regained. Used for other purposes
D_{72}	F	9.0	30 ●	0.5	262 △	0.0	Typical symptoms. Autopsy—no ulcers or hemorrhages in intestinal tract
D_{73}	F	10.0	55 ●	0.0	242 ●	3.2	No symptoms. Employed for other purposes

○ Received 1 yeast cake daily for last 19 days on C-U diet.
□ Received 1 to 4 yeast cakes daily for loss of hair.
▲ Received 1 yeast cake daily for last 9 days on C-U diet.
● Received 1 yeast cake daily.
■ Gave birth to five pups. All died in few days

In the experiments that follow immediately, the food ingested had the composition noted below:

Composition of food expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Cottonseed oil	5.0	
(Or later butter fat)	5.0	
Yeast		
Total		0.30+

Salt mixture—2 per cent of total food.

Experiment 18—group I. Experiments in which dogs were cured of the disease and were then maintained for extended periods of time. In table 21 detailed data relative to this group may be found.

Discussion. From the results of table 21 it is quite evident that with

this group of animals comprising 18 dogs butter fat is capable of curing the diseased condition under discussion, and further that the dogs thus cured are enabled to live upon a diet containing butter fat for extended periods. In this connection it must be emphasized that all of these dogs would have died in a few days without such addition of butter fat. When

TABLE 22

Results of experiment 18—group II

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF WEIGHT	PERIOD ON BUTTER FAT	LOSS OF WEIGHT	REMARKS
		kilos	*days*	*kilos*	*days*	*kilos*	
D_{27}	F	11.1	22*	1.1	23†	0.9	Diarrhea. Inflamed mouth. Autopsy —lesions characteristic
D_{32}	F	9.0	20‡	1.0	32§	0.5	Died in tetany. No diarrhea or mouth lesions. Autopsy—typical intestinal lesions
D_{38}	M	12.2	22¶	0.8	64§	1.7	Inflamed mouth
D_{29}	F	7.5	17‡	0.3	81§	2.1	Atypical symptoms. Mouth very pale
D_{40}	F	9.3	11*	0.4	38§	2.7	Atypical symptoms. At autopsy no changes except emaciation
D_{41}	F	10.9	42‖	1.1	38§	1.5	Diarrhea. Inflamed mouth. Convulsions
D_{46}	M	13.1	15¶	0.3 gain	55§	5.2	Diarrhea. Emaciation. Mouth pale. No lesions in intestinal tract
D_{67}	M	7.8	17†	0.1	63 #	1.7	No typical symptoms. Mouth pale. No lesions in intestinal tract
D_{69}	M	5.7	10†	0.6	34†	0.2	No typical symptoms. Mouth pale. No lesions in intestinal tract

* Received 2 yeast cakes daily for last 5 days.

† Received 1 yeast cake daily.

‡ Received no yeast.

§ Received 2 yeast cakes daily.

¶ Received 2 yeast cakes daily for last 9 days.

‖ Received 2 yeast cakes daily for last 32 days.

Received 1 yeast cake daily for 44 days, then 2 yeast cakes daily for 6 days, then 3 yeast cakes daily for 23 days.

this fact is recognized the effect of butter fat as a curative agent is truly remarkable.

Of the eleven animals that died six manifested characteristic symptoms and in general typical lesions at autopsy. Three dogs died of pneumonia and without symptoms that could be attributed to the diet. Two dogs died with atypical symptoms and the cause of death is not obvious.

Of the seven dogs that lived, six were employed for other experiments.

One dog is still living and was maintained on the butter diet from November 21, 1922 until June 16, 1927, a period of more than 4½ years. This animal was carried through to determine the length of time possible for a dog to live upon this diet. When the investigation closed in June 1927, there was every indication that the dog would have been maintained to old age.

A study of the results will reveal that yeast added to the diet had little or nothing to do with the reëstablishment and maintenance of normal nutritive conditions. If yeast did possess preventive properties there was certainly ample opportunity for it to be manifested in several of these

TABLE 23

Results of experiment 18—group III

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF WEIGHT	PERIOD ON BUTTER FAT	LOSS OF WEIGHT	REMARKS
		kilos	*days*	*kilos*	*days*	*kilos*	
D_{24}	M	8.6	95*	0.4	4†	0.7	Typical symptoms
D_{25}	M	11.3	59†	1.7	5†	0.2	Typical symptoms
D_{29}	F	8.2	20‡	0.2	2†	0.3	Typical symptoms
D_{31}	F	9.0	18‡	0.2	2†	0.8	Typical symptoms
D_{34}	M	10.9	26‡	0.7	2†	0.0	Typical symptoms
D_{37}	F	9.7	39‡	0.2	8†	1.1	Typical symptoms
D_{45}	F	11.3	18‡	1.3	14†	1.1	Typical symptoms
D_{54}	F	7.7	45§	0.5	5†	0.2	Typical symptoms
D_{56}	M	10.5	25‡	0.2	4†	0.0	Typical symptoms
D_{57}	F	9.0	37‡	0.1	4†	0.1	Typical symptoms
D_{59}	F	9.0	45‡	0.1	2†	0.1	Typical symptoms
D_{63}	F	8.4	32¶	2.0	4†	0.4	Typical symptoms
D_{64}	F	9.0	58‖	0.6	7†	0.4	Typical symptoms

* Received 1 or 2 yeast cakes daily for last 83 days.
† Received 2 yeast cakes daily.
‡ Received no yeast.
§ Received 2 yeast cakes daily for last 24 days.
¶ Received 2 yeast cakes daily for last 10 days.
‖ Received 2 yeast cakes daily for last 29 days.

experiments when yeast in the quantity of two cakes per day was given. It is, however, our distinct impression that yeast is of value in preventing, or even, perhaps, in alleviating the skin rash, which has so persistently attacked some of our animals in previous experiments.

Conclusion. Butter fat is capable of curing this disease and is able to maintain cured animals for extended periods of time.

Experiment 18—group II. Experiments in which dogs were cured of the disease but in which butter fat failed to maintain them for prolonged intervals. The general procedure was the same as that of group I and the results are tabulated in table 22.

Conclusion. The results of group II point very clearly to the efficacy of butter fat as a curative agent, but also indicate with equal clarity its inability to maintain these animals in good nutritive condition for prolonged intervals.

Experiment 18—group III. Experiments in which butter fat failed to cure. The animals comprising this group were treated in every way comparable to those of the two former groups. The detailed results may be found in table 23.

Conclusion. Butter fat failed to effect a cure of the disease in these thirteen animals.

Three possibilities for failure are obvious: *a.* The disease had progressed too far for cure. *b.* In view of the fact that these sick animals find difficulty in retaining butter fat an insufficient quantity may have been absorbed. *c.* The butter fat used may have lost its potency.

Is fat soluble vitamin A of butter the effective factor? In attempting to explain the action of butter in preventing and curing this experimental disease one naturally queries whether its content in fat soluble vitamin A is the effective agent. To gain evidence relative to this point it is logical to assume that if this vitamin is the effective agent an active preparation of cod liver oil should be capable of preventing and curing the disease. In accord with this hypothesis experiments have been carried through to test the preventive and curative properties of a brand of cod liver oil known from experiments in the Department of Pediatrics to be active.

Experiment 19. Will cod liver oil prevent the disease? The plan of these tests was similar to that of previous experiments on the protective action of butter fat. Four tests comprise this experiment. In two of them cod liver oil was the only fat fed (test B); in the other two tests cod liver oil replaced two-thirds of the cottonseed oil of the diet (test A).

Test A—Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Cod liver oil	3.3	
Cottonseed oil	1.6	
2 Yeast cakes		
Total		0.30+

Salt mixture—2 per cent of total food.

Protocols of dogs 42 and 55

DOG	SEX	INITIAL WEIGHT	LENGTH OF LIFE	CHANGE IN WEIGHT	REMARKS
		kilos	*days*	*kilos*	
42	M	8.9	63	+1.5	Diarrhea. Foul mouth
55	F	7.0	41 114*	−0.4	Diarrhea. Foul mouth

* Dog 55 was maintained for 41 days on the above diet when the typical symptoms appeared. In order, if possible, to save the dog, it was given butter fat for 6 days in place of the cottonseed oil Then cod liver oil replaced the butter fat so that the dog received 5.0 grams per kilo of cod liver oil per day.

Conclusion. It is obvious that cod liver oil will not prevent the appearance of the disease when the oil is administered in the quantities here given. It is quite probable, however, that it is not entirely devoid of protective action since life was maintained for a somewhat longer period than is usual when cottonseed oil furnished the entire fat requirement.

Test B—Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Cod liver oil	5.0	
2 Yeast cakes		
Total		0.30+

Salt mixture—2 per cent of total food.

Protocols of dogs 60 and 68

DOG	SEX	INITIAL WEIGHT	LENGTH OF LIFE	LOSS IN WEIGHT	REMARKS
		kilos	*days*	*kilos*	
60	F	16.4	57	4.0	Death in convulsions. No typical symptoms or lesions
68	F	8.5	88	0.1	Diarrhea and foul mouth

Conclusion. The failure of these animals to survive for prolonged periods of time with the inclusion of cod liver oil in the diet indicates with certainty that cod liver oil is not an effective protective agent against this experimental disease.

Will cod liver oil cure the disease? In order to ascertain whether cod liver oil possesses any curative action two dogs were placed upon the

above modified C-U diet until definite symptoms appeared when cod liver oil was given in place of the cottonseed oil in equivalent amounts.

Protocols for dogs 54 and 62

DOG	SEX	INITIAL WEIGHT	PERIOD ON C–U DIET	PERIOD ON COD LIVER OIL	LOSS OF WEIGHT	REMARKS
		kilos	*days*	*days*	*kilos*	
54	F	8.8	34*	32†	1.9	Typical death
62	M	7.5	37‡	7†	0.4	Typical death

* Received 1 yeast cake for last 20 days.
† 2 yeast cakes daily.
‡ No yeast given.

Dog 54 lived for a considerable period after being placed upon cod liver oil and from the length of life one might suspect a considerable degree of curative action. It may be that cod liver oil does possess a certain amount of the effective substance but the evidence for it is weak. The protocol unfortunately does not give a true picture of this dog's condition. As a result of living upon the C-U diet, symptoms appeared which consistently persisted in spite of the administration of cod liver oil. The long life of this animal is, perhaps, best explained by the fact that very little food was ever consumed on any one day after the appearance of symptoms. The oil and yeast, however, were fed separately to the dog daily.

With dog 62 the experiment with cod liver oil is a frank failure, as the cod liver oil was given at the first hint of symptoms. That the administration of the oil was not too late is attested by the fact that the dog ate all its food from the beginning of the experiment until two days before death when the oil and yeast were given separately.

The results of experiments with cod liver oil, both as preventive and curative agent, were so little encouraging as to warrant discontinuance of this method of investigation.

Conclusion. Cod liver oil possesses little, if any, curative action against this experimental disease in dogs.

From the fact that cod liver oil, demonstrably active as to its content in fat soluble vitamin A, possesses to an insignificant degree, if at all, protective or curative properties relative to the disease under discussion, it seems rational to conclude that vitamin A plays little part in the protective and curative properties demonstrated for butter fat.

The instability of the effective agent in butter fat. In our efforts to demonstrate the curative properties of butter fat we have been surprised at the variability in the potency of this substance. Several years ago this fact was emphasized in an experiment in which several dogs had lived for a

comparatively long period upon a diet containing butter fat. Suddenly in all instances typical symptoms appeared and several of the dogs died. At this time a particular brand of butter was being fed. Some of the dogs were saved by administration of freshly-made butter.

This experience led us to secure butter from a known source of supply. A large quantity (1500 pounds) was laid down in July in Vermont, shipped to New Haven and kept in cold storage, being withdrawn as needed. Our various experimental animals were fed from this source of supply only. Curative and maintenance experiments were successful with this butter for a period of 11 months when apparently it had lost so much of its protective action as to be ineffective. On the other hand, butter bought on the open market was effective where the storage butter failed.

Under exactly similar circumstances the following year another large shipment of butter from Vermont was received and in approximately 9 to 10 months this butter also became inefficient as a curative agent. From these two experiences we have come to the conclusion that butter kept for long periods gradually loses its protective action. Since then we have endeavored to obtain butter as freshly made as possible and our success has been uniformly good.

In order to show the contrast of length of life of dogs on a diet with butter kept in cold storage for a considerable length of time, and those receiving different brands of presumably fresh butter, attention is directed to some of the dogs in experiment 18—group I.

DOGS FED STORAGE BUTTER	LENGTH OF LIFE	DOGS FED VARIOUS SAMPLES OF BUTTER	PERIOD ON DIET
	days		*days*
D_{18}	240	D_{33}	1689 (still living)
D_{21}	151	D_{42}	111*
D_{26}	140	D_{61}	205*
D_{50}	162	D_{65}	470*
D_{52}	149	D_{66}	705*
D_{58}	126	D_{71}	243*
D_{72}	262	D_{73}	242*

* Then used for other purposes.

It is quite probable that the failure of many dogs in experiment 18—group II to maintain themselves is explained in the same way.

More direct evidence of the point under discussion is furnished by the following experiment in which three dogs were fed on butter fat which previously had possessed curative properties but later had lost them.

Dog 35 was fed on one sample of butter.

Dogs 44 and 48 were fed with a different butter sample. The diet had the following composition:

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Butter fat	5.0	
Total		0.30+

Salt mixture—2 per cent of total food.

Experiment 20.

TABLE 24

DOG	SEX	INITIAL WEIGHT	LOSS OF WEIGHT	LENGTH OF LIFE	REMARKS
		kilos	*kilos*	*days*	
D_{44}	M	9.2	2.4	76*	Diarrhea. Convulsions. No mouth lesions
D_{45}	M	8.9	0.0	34†	Inflamed mouth. Convulsions. Lesions in intestine
D_{35}	F	5.7	1.7	43‡	Ulcerated mouth

* Received 2 yeast cakes daily for last 65 days.
† Received 2 yeast cakes daily for last 12 days.
‡ Received 2 yeast cakes daily for last 36 days.

Conclusion. From the results of this experiment it may be concluded that the samples of butter employed had lost a major portion, at least, of the effective agent, since these specimens possessed neither protective or curative properties. Whatever its nature, the effective agent in butter fat may be lost, or at least greatly diminished in amount, even during maintenance in cold storage.

The relationship between the effective agent and carotinoids. When it became clear that our second shipment of Vermont butter had lost its potency, a chance observation revealed the fact that the characteristic yellow color of the butter had also almost entirely disappeared. This coincidence led us to test the possibility whether there is a relationship between the pigment of butter and the effective agent. The coloring matter of butter, carotin, is widely distributed in nature and thus far no definite physiological function has been ascribed to it. The idea that carotin may serve a useful purpose in nutrition is by no means novel, for even recently Steenbock (5) and his associates thought that it might be identical with the fat soluble vitamin A. This, however, was later disproved. The whole subject of the chemistry, distribution, etc., of carotin

has been so well summarized by Palmer (6) that a detailed account here is superfluous.

Does egg yolk contain the effective factor? Egg yolk contains a relatively large quantity of yellow pigment belonging to the group of carotinoids, the so-called xanthophylls, and a smaller quantity of carotin (6). In addition, however, it contains the fat soluble vitamin A which may complicate any endeavor to differentiate between the effects of these two constituents. In general, the carotinoids and the fat soluble vitamin A usually, although not necessarily, accompany each other. For our purpose, however, the presence of the fat soluble vitamin A is probably not a com-

TABLE 25

Results of experiment 21

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF WEIGHT	PERIOD ON EGG YOLK DIET	GAIN IN BODY WEIGHT	REMARKS
		kilos	*days*	*kilos*	*days*	*kilos*	
D_{73}	F	13.2	16	1.4	434	1.8	No symptoms on egg yolk diet. Used for other purposes
D_{74}	M	8.2	57	0.2	102	0.4	Died suddenly with no symptoms. No lesions
D_{75}	M	10.2	74	0.2	4	0.0	Typical symptoms. Bloody diarrhea. Foul mouth
D_{76}	M	10.0	51	1.2	15	0.3	Typical symptoms. Bloody diarrhea. Foul mouth
D_{77}	M	8.0	20	0.3	81	0.1	No symptoms. Death atypical
D_{82}	F	8.1	41	0.3	83	0.8	No symptoms on egg yolk diet. Used for other purposes

Note: All of these animals received 1 or 2 yeast cakes per day throughout the experiment.

plicating factor since it has been demonstrated previously that this vitamin is not the effective factor in butter fat.

Experiment 21. The method of attack in this problem has been that previously employed, namely, the induction of the symptoms by feeding the C-U diet and then the attempt to cure by the supplementary administration of egg yolk. The eggs were hard boiled and if the dog would not eat voluntarily the yolks were force fed. The usual dosage was the yolks of three eggs daily.

The results of this experiment may be found in table 25.

From the table it is apparent that egg yolk cured four out of six of these dogs and maintained them for reasonable periods of time.

Conclusion. Egg yolk contains the agent effective in curing our experi-

mental disease. Although positive proof is lacking the impression was gained that fresh eggs are more efficient than cold storage eggs.

The curative properties of carrots. The experiment with egg yolk was so encouraging as to lead us to extend the investigation to the carrot which contains a relatively large quantity of carotin. Egg yolk coloring matter is largely xanthophyll according to Palmer (6), only a small amount of carotin being present. Since xanthophyll and carotin are so closely related chemically, xanthophyll being regarded as carotin dioxide, it may be possible that in the absence of one the other may fulfill its function.

In the following experiment the curative action of carrots has been tested.

Experiment 22.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Cottonseed oil	5.0	
1 Yeast cake daily		
Total		0.30+

Salt mixture—2 per cent of total food.

When the symptoms of the disease appeared the cottonseed oil was replaced by 150 grams of boiled *unpeeled* carrots.

The results are detailed in table 26.

The five experiments detailed in table 26 show clearly that boiled unpeeled carrots are capable of curing the experimental disease and are then able to maintain the dogs in excellent nutritive condition for extended periods of time. It is noteworthy that a failure to cure the disease has not been experienced. In this respect, therefore, the carrot is much more effective than butter fat. Whether this difference is due to the greater content of the effective agent in the carrot or whether the better results obtained are due to its greater stability in the carrot is uncertain.

A study of the table above will show that although the dogs were maintained in excellent nutritive condition they gained very little in weight or else suffered a decided loss. The loss in weight occurred early after the administration of carrots and then the weight became stabilized. It is quite probable that the loss in weight may be attributed to the lower calorific intake since carrots entirely replaced the cottonseed oil so that the total calorific intake suffered a distinct reduction. It should also be remembered that these animals were maintained on a low nitrogen intake, which together with the diminished calorific values would naturally tend

to a lowering of body weight. Thus the conditions provided a severe test for the curative agent.

In line with this reasoning is an experiment with dog D_{65}, not detailed above. This animal had been maintained for a period of 470 days on butter fat and was in excellent condition except that it had become almost hairless. Its body weight had remained stationary for a long time. It was placed upon a ration identical in every respect to its usual diet except

TABLE 26

Results of experiment 22

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF BODY WEIGHT	PERIOD ON CARROTS	CHANGE IN WEIGHT	REMARKS
		kilos	*days*	*kilos*	*days*	*kilos*	
D_{78}	M	8.9	22	0.2	244	0.4 gain	No symptoms of any sort on carrot diet. Dog maintained in excellent condition. Used for other experiments
D_{79}	F	8.5	61	0.0	193	0.6 gain	No symptoms of any sort on carrot diet. Dog maintained in excellent condition. Used for other experiments
D_{80}	F	10.5	64	0.5 gain	137	0.6 gain	No symptoms of any sort on carrot diet. Dog maintained in excellent condition. Used for other experiments
D_{81}	F	9.2	33	0.2	135	3.2 loss	No symptoms on carrot diet except rapid growth of several large wart-like growths on lips which mechanically interfered with eating. Dog killed. At autopsy no lesions were found
D_{71}	F	14.2	26	0.3	406	4.5 loss	No symptoms of any sort on carrot diet. Dog maintained in excellent condition. Used for other experiments

that 150 grams of carrots replaced the butter fat. Immediately it began to lose weight. It was maintained on the carrot diet for 101 days during which period, although in excellent condition, it had lost 1.1 kilos. Here there can be no doubt that loss of weight was directly related with lowered intake of calories.

The efficiency of carrots has been established. No attempt has been made to determine the exact quantity of carrots necessary to cure the disease or to maintain a dog on the C-U ration. The quantity given was

chosen arbitrarily and is certainly sufficient. Whether greatly diminished quantities would be equally efficient is unknown.

There is one strange fact with respect to the behavior of diseased dogs that is worthy of note. When attempts are made to cure the disease with butter fat, the animals show a great reluctance to eat the fat and even when it is placed in the throat fight against swallowing it. With carrots, however, no such attitude is to be observed. Instead, they take the carrots readily, and when they have reached the stage of voluntary eating, the carrots are greadily devoured.

Conclusion. Cooked carrots are very effective in curing the disease, since no failures have been experienced. They are also capable of maintaining such cured dogs for prolonged periods of time in an excellent state of nutrition when the rest of the diet consists of the C-U ration only.

Experiment 23. The curative effect of a lard extract of carrots. In the attempt to arrive a little more closely at the effective agent in carrots a few experiments have been carried through with a lard extract of carrots. Lard heated just sufficiently to render it liquid was mixed with finely ground air-dried carrots, and allowed to stand for 8 days. The proportion of lard to carrots was such that 50 grams of lard were mixed with the equivalent of 15 grams of fresh carrots. The mixture was finally extracted four times with ether in the cold. The ether was then removed by evaporation at room temperature. The extract was fed to dogs in the dosage of 5 grams per kilo of body weight. It was bright yellow, and probably contained, in addition to carotin, significant quantities of the fat soluble vitamin A.

The usual plan of procedure was followed: dogs were given the modified C-U diet until symptoms appeared, then the cottonseed oil was replaced by the lard extract.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Cottonseed oil	5.0	
1 or 2 Yeast cakes per day		
Total		0.30+

Salt mixture—2 per cent of total food.

The results of this experiment may be found in table 27.

The tabulated results of experiment 23 clearly indicate that the lard extract of carrots contains the effective factor since in the three animals tested it was capable of restoring them to a normal condition after the

appearance of symptoms due to the disease-producing C-U diet. After these animals had been maintained on carrot extract for a period sufficiently

TABLE 27

Results of experiment 23

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF WEIGHT	PERIOD ON CARROT EXTRACT	GAIN IN WEIGHT	SECOND PERIOD ON DISEASE PRODUCING DIET	REMARKS
		kilos	*days*	*kilos*	*days*	*kilos*	*days*	
D_{78}	M	9.0	56	0.0	56	0.8	49	No symptoms while on carrot extract. All food eaten
D_{80}	F	10.0	64	0.0	61	0.8	14	No symptoms while on carrot extract. All food eaten
D_{95}	M	5.9	23	0.3	25	0.2	26	No symptoms while on carrot extract. All food eaten

TABLE 28

Results of experiment 24

DOG	SEX	INITIAL WEIGHT	PERIOD ON DISEASE PRODUCING DIET	LOSS OF WEIGHT	PERIOD ON CAROTIN	PERIOD ON BUTTER	CHANGE IN WEIGHT	SECOND PERIOD ON DISEASE PRODUCING DIET	REMARKS
		kilos	*days*	*kilos*	*days*	*days*	*kilos*	*days*	
D_{78}	M	9.8	49	0.0	21	7	0.0	56	Animal recovered. Used for other experiments
D_{88}	M	9.1	91	0.2	11	9	0.1 gain	17	Animal recovered. Used for other experiments
D_{91}	M	11.8	50	0.6	11	10	0.0	20	Animal recovered. Used for other experiments
D_{95}	M	5.8	26	0.0	12	6	0.2 gain	26	Animal recovered. Used for other experiments
D_{96}	M	5.7	46	0.4	11	6	0.0	52	Animal recovered. Used for other experiments
D_{98}	F	5.0	16	0.3	13	0	0.3 loss		Dog died of pneumonia
D_{99}	M	8.3	18	0.0	15	20	0.5 loss		Dog killed for other purposes
D_{80}	F	10.8	119	0.5 gain	8	6	0.3 loss	6	Dog recovered. Used for other purposes

long to demonstrate the effectiveness of the extract, cottonseed oil was substituted for the carrot extract in order to make certain that their recovery was not due to some extraneous circumstance or factor. It is quite

apparent from table 27 that the restoration to a normal state must be ascribed to the carrot extract for when placed again on the modified C-U diet all three of these animals developed typical outspoken symptoms of the disease. It is of interest in this connection to note the different periods of time essential to develop the symptoms a second time. In dogs 78 and 95 the disease appeared in about the same period as in the first trial; with dog 80 it developed much more rapidly. This dog was apparently more sensitive during the second trial.

Conclusion. The bright yellow lard extract of carrots contains the agent effective in alleviating the symptoms of this experimental disease.

The effectiveness of crystallized carotin. The experience with butter fat, egg yolk, carrots and the lard extract of carrots justifies the assumption that the effective agent, if not a carotinoid itself, has at least a similar distribution and is closely associated with it. A decisive answer to the question could be gained only by trial with crystallized carotin. The results of such tests may be found in table 28. A portion of the carotin was kindly furnished by Prof. J. C. Drummond of University College, London; the remainder was prepared by us according to Drummond's directions. It was recrystalled 6 times. We deviated from Drummond's procedure in the fact that for crystallization purposes an atmosphere of carbon dioxide was employed instead of one of nitrogen.

The crystalline product was fed either dissolved in cottonseed oil or as a solid enclosed in ground meat. It was administered separately from the remainder of the food in order to make certain that it was entirely ingested.

Experiment 24. The plan of this experiment followed the usual procedure. Dogs were given the modified C-U diet until definite symptoms appeared; then carotin was given in the dosage of 5 mgm. per day.

Composition of diet expressed in grams per kilo of body weight

	FOOD SUBSTANCE	NITROGEN
Meat	4.1	0.15
Cracker meal	8.8	0.15
Cottonseed oil	5.0	
1 or 2 Yeast cakes per day		
Total		0.30+

Salt mixture—2 per cent of total food.

A survey of table 28 demonstrates that pure crystallized carotin in doses of 5 mgm. per day per dog is capable of causing the symptoms of the disease under discussion to disappear. It will be noted that in all but one instance butter fat was fed following the administration of carotin. This was done to conserve our supply of carotin. Butter, however, was not

administered until it was certain that the animal had been restored to an apparently normal condition and butter fat was supplied merely for maintenance after such restoration. The butter fat played no rôle in the restoration.

Generally when a dog gives definite symptoms of the disease, the administration of carotin for 10 to 15 days in the dose indicated above is sufficient to bring back the animal to a normal condition, as indicated by disappearance of symptoms, recovered appetite, etc. In several cases carotin plus a small quantity of meat and at times one yeast cake constituted the only food ingested for several days. Suddenly the animal resumed eating and all food was greedily devoured.

To demonstrate the effectiveness of the restoration to normal, these dogs were not allowed to continue for a long period on butter fat by which, perhaps, they might gain a resistance to the disease, but on the contrary, were subjected to another severe test, namely, again placed upon the disease-producing diet. Even under these circumstances, the data in table 28 show clearly that, in all but one instance, dog D_{80}, a considerable period elapsed before the symptoms appeared a second time. It is apparent that the dogs had developed a marked degree of resistance. This experiment also demonstrates that carotin was the effective agent in the restoration to health since the change to cottonseed oil was followed by the reappearance of the disease. In this connection it should perhaps be pointed out that throughout our whole experience not a single animal has ever made a spontaneous recovery after definite symptoms of the disease had developed.

We have records of a number of experiments in which carotin failed to save dogs suffering with the disease. These results, however, are not pertinent in this connection since in some of these tests carotin had been fed for two or three days only and its failure can be attributed to the fact that the disease had progressed too far to make it possible to restore these dogs to a normal condition. In other dogs carotin was not retained, everything reaching the stomach being immediately vomited.

In ascribing to carotin the rôle of effective factor in the alleviating of the disease symptoms one does so with the reservation of the possible presence of some unknown contaminating substance. If carotin itself is not the effective agent, but the action is due to some included compound, the latter must be present in minute quantities and must be an exceedingly potent substance. Moreover, it hardly seems probable that it would possess the same distribution, solubilities, fractional crystallization, etc., of carotin. Until the presence of some such remarkable substance has been demonstrated one is justified in ascribing the remedial properties to carotin[3] itself.

[3] In considering carotin as the effective agent in this investigation an apparent contradiction may be found in the fact that fresh pig's liver contains the effective

Great caution in drawing any final conclusion is prompted by the recent developments with regard to the antirachitic substance developed by irradiation of cholesterol. It has been clearly established that it is not the latter but some contaminating compound, probably ergosterol, present in exceedingly small quantities that is the "provitamin" capable of becoming activated by ultra-violet rays. The ergosterol cannot be separated effectively from cholesterol by ordinary processes of recrystallization.

In 1925 we (8) published a short resumé of part of the results detailed herein. Since then Goldberger (9) and his associates have stated that in the treatment of so-called black tongue in dogs butter fat failed as a preventive substance just as it has failed, in their hands, in the treatment of human pellagra. Moreover, they further state that "this vegetable (the carrot) contains the black tongue preventive factor, but in relatively small amounts (10)." Carrots likewise failed to prevent human pellagra.

The discrepancies between the results of Goldberger and his associates and our own are so great as to prohibit attempts at explanation. Moreover, the character of the food they fed and their experimental conditions are so different from our own that comparison of the two investigations is difficult, especially since no detailed report of their experiments has yet appeared. We shall be content, therefore, merely to call attention to the apparent lack of agreement between the results of the two investigations.

Conclusion. Carotin prepared from carrots is capable of restoring to apparent health dogs suffering from the disease described by Chittenden and Underhill.

SUMMARY OF CONCLUSIONS

When butter fat is fed to dogs suffering from the deficiency disease described by Chittenden and Underhill in 1917 these animals are quickly restored to a normal condition and may be maintained in health for prolonged periods.

Under similar circumstances cod liver oil, known to contain active fat soluble vitamin A, fails to restore these diseased dogs. Evidently this vitamin is not the potent factor in butter fat.

The effective factor in butter fat is apparently quite labile for the potency is diminished by maintenance in cold storage during a period of 9 to 11 months.

The presence of the potent factor in butter fat is associated with the color of the butter. As the color decreases the potency is diminished.

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agent. According to Palmer (7) pig's liver does not contain carotin. On the other hand, this statement of Palmer must be erroneous for Prof. J. C. Drummond assures us from his own work that pig's liver does contain carotin. This apparent discrepancy is thereby clarified.

Egg yolk contains the effective factor but is apparently less potent than butter fat.

Boiled unpeeled carrots are even more effective in curative properties than is butter fat.

Lard extracts the potent agent from carrots.

It would appear that there is a direct relationship between the presence of carotinoids in the substances tested and the potent factor.

Crystallized carotin in relatively small doses is capable of curing the diseased condition.

Finally, these experiments lend support to the conception that naturally occurring pigmentary substances in foodstuffs play a rôle in nutritive processes.

BIBLIOGRAPHY

(1) Chittenden, R. H. and F. P. Underhill. This Journal, 1917, xliv, 13.
(2) Wheeler, G. A., J. Goldberger and M. R. Blackstock. Pub. Health Rept., Washington, D. C., 1922, xxxvii, 1063.
(3) Cary. Journ. Amer. Vet. Med. Assoc., 1920, lxvi, 609. Quoted from Goldberger.
(4) Wheeler, G. A., J. Goldberger and R. D. Lillie. Pub. Health Rept., Washington, D. C., 1926, xli, 297.
(5) Steenbock et al. Cf. Palmer. Carotinoids and related pigments, 1922, 269, where this aspect of the problem is discussed.
(6) Palmer. Carotinoids and related pigments, 1922.
(7) Palmer. Loc. cit., 139.
(8) Underhill, F. P. and L. B. Mendel. Pub. Health Rept., Washington, D. C., 1925, xl, 1087.
(9) Goldberger, J., G. A. Wheeler and R. D. Lillie. Pub. Health Rept., Washington, D. C., 1926, xli, 300.
(10) Goldberger, J. and G. A. Wheeler. Loc. cit., 1927, xlii, 1303.

Acknowledgments. This investigation has been made possible through grants from the Bache Fund of the National Academy of Sciences, the Russell Sage Institute of Pathology, the Loomis Research Fund of the School of Medicine, Yale University, and by coöperation with Field Studies in Nutrition, United States Public Health Service.

Acknowledgment is also made of the coöperation of Dr. E. G. Gross, Dr. L. J. Bogert and Dr. F. I. Peterman in the conduct of the experiments. We are particularly indebted to Dr. Robert A. Lambert for studies of the morphological pathology involved in the disease.

It is a pleasure to acknowledge the generosity of the Yale University Press in furnishing funds to meet the expenses attendant upon the reproduction of the colored plates contained in this report.

STUDIES ON THE KNEE JERK

II. The Effect of Moderate Doses of Morphine Sulphate on the Reflex Excitability of the Spinal Cord

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Received for publication September 21, 1927

Several years ago when Collins and Tatum (1) first reported their work on the genesis of morphine tolerance in animals, the question was raised as to whether the symptoms of excitability seen by them were due to the primary effects of the drug, masked by early depression, or whether this alleged stimulation developed secondarily after a primary period of depression. No experiments having been performed specifically on the comparative effects of morphine on the cord and higher centers respectively, it was assumed on the basis of previous work that whereas morphine depresses the higher centers, it exerts at the same time a strychnine-like action on the spinal cord. According to the prevalent view, a primary excitation of the cord accompanies the powerful central depression and is masked by the latter; for among other things, the knee jerk is found, at the time, to be quite brisk, perhaps even more brisk than prior to the injection of the drug. When the central depression diminishes, the excitatory symptoms presumably step into the foreground and apparently outlast the former.

While recording the knee jerk in a barbitalized dog, it seemed desirable to quiet the partially anesthetized and restless animal by a small dose of morphine. A small dose of morphine sulphate ($\frac{1}{4}$ gr.) was injected subcutaneously. As the injected morphine took effect, the excitability of the cord, judged by the height of the registered kick, showed a progressive depression. When attention was called to this result, and others of similar nature, objection was raised to the data obtained on the ground that the depression was obtained from an animal under an anesthetic. This objection did not seem valid, since under identical conditions, minute quantities of strychnine sulphate (1 mgm.) administered subcutaneously or intravenously effected an almost immediate and marked increase in cord excitability. In order, however, to obtain final analysis of the action of

[1] Aided by a grant from the Frank Billings Medical Club, Chicago, Ill.

morphine on the reflex excitability of the cord, a variety of experiments was performed, the results of which form the body of this report.

In this connection it might be pointed out that Sloan (2) succeeded in maintaining alive parathyroidectomized dogs by the timely use of morphine sulphate. Injection of morphine sulphate during an attack of tetany was followed by a disappearance of the neuromuscular phenomena so characteristic of this syndrome. Since parathyroid tetany has its origin, in *part*, in the cord and since this tetany can be abolished by morphine we were inclined to adhere to our hypothesis that the primary action of morphine on the cord, given in moderate doses ($\frac{1}{2}$ to 18 gr.), is in the direction of depression.

METHODS. The evidence submitted that morphine in moderate doses produces either a depression of the cord, or no effect at all, was obtained from direct quantitative measurement of the cord excitability by a special knee jerk apparatus described in a previous paper (3). It will be recalled that this apparatus automatically delivers a constant blow at a definite time interval (7 seconds) and that each kick is recorded. Our judgment of the effect of any drug or procedure is based on the record of a great number of knee jerks as compared with a long series of knee jerks taken during a control period.

Most of the work was carried out on dogs under various experimental procedures. The initial experiments were carried out on dogs under Na barbital anesthesia (225 to 250 mgm. per K) in order to isolate the cord from the higher centers and during the period of anesthesia a dose of morphine was given ($\frac{1}{4}$ to 18 gr.). The effect of morphine was also studied in barbitalized dogs whose cords had been transected a few hours previously. In other dogs the effect of morphine was studied at various intervals following transection of the spinal cord in the region of the 12th thoracic vertebra. One of the animals had had its cord transected two years previously. Modifications of the usual type of procedure will be mentioned in the course of the discussion of the results. These modifications were introduced as an aid in the interpretation of the results.

RESULTS. 1. *The effect of morphine sulphate ($\frac{1}{4}$ to 18 gr.) subcutaneously injected on the intact cord of the barbitalized dog.* Na barbital (225 to 250 mgm. per K) was given intraperitoneally forty-five minutes previous to any experimental procedure, and by this time the animal was under uniform anesthesia. The animal was arranged for taking knee jerks; and records were taken of the knee jerks and blood pressure. A control period was taken for about an hour and under suitable conditions it was possible to secure a record of approximately four hundred jerks in which no single jerk varied from any other single jerk more than one millimeter in amplitude. (The apparatus could be so adjusted that any desired amplification could be had. Usually the recorded knee jerk was about 80 mm. in height.)

Fig. 1. Showing the effect of subcutaneous injection of morphine sulphate (2½ grains) on the knee jerk of a 16.6 kgm. dog under barbital Na anesthesia (2 grams given intraperitoneally).

A = Normal knee jerk. *B* = Record taken 6 to 7 minutes after taking record *A*. At *1* injection of 2½ grains (150 mgm.) of morphine sulphate. *C* = The height of the knee jerk 35 to 40 minutes after the injection of the morphine. *D* = The extent of the knee jerk 10 minutes later. *E* = 10 minutes after taking *D*. *F* = 10 minutes after taking *E*.

Fig. 2. Showing the effect on the knee jerk following the intravenous injection of ½ grain morphine sulphate (30 mgm.) into a 20.5 kgm. dog whose cord had been transected in the lower thoracic region under barbital Na anesthesia (4.6 grams barbital Na given intravenously). Blood pressure record uppermost in tracing.

A = Transection of the cord at *1*. *B* = At *2* intravenous injection of 30 mgm. morphine 35 minutes after transection of the cord. *C* = Record of the knee jerk 35 minutes after *B*. Note almost immediate diminution of the knee jerk after the injection of morphine. In the course of the next ½ hour the progressive diminution, *C*, of the knee jerk resulted in an almost complete abolition. Strychnine sulphate similarly injected in doses of 1 mgm. or less has precisely the opposite effect.

Fig. 3. Showing the effect of the subcutaneous injection of 1 grain morphine sulphate in a dog whose spinal cord had been transected in the lower thoracic region 12 days previously. Observations made on the unanesthetized animal.

A = Irregular character of the knee jerk prior to the injection of the morphine. During this period spontaneous movements were present in the hind limbs. Flexion, extensor, and tail reflexes could be elicited with the greatest ease as a result of slight mechanical stimulation. *B* = Knee jerk record at the time of the subcutaneous injection of 1 grain of morphine sulphate. *C* = Taken 25 minutes following administration of the morphine. *D* = Taken 1¾ hours after administration. Flexion, extension, and tail reflexes were obtained only slightly following powerful mechanical stimulation. *E* = 45 minutes after *D*. At this time clamping hemostat on leg or tail failed to elicit reflex responses.

Fig. 4. Showing the effect of morphine sulphate in releasing the reflex center of the cord concerned in the knee jerk from the influence of various afferent impulses which without morphine affect the limb or center so that the extent of the knee jerk is greatly reduced in height.

Unanesthetized dog. Cord transected 14 days previously. Reflexes posterior to the transection very brisk. Numerous spontaneous movements of tail and legs.

A and *B* are representative tracings of the character of the response for 1 hour, 15 minutes before the subcutaneous administration of 1 grain of morphine given at *B*. *C* = Knee jerk one hour after injection of the morphine showing higher and more regular response. *D* = Record 15 minutes later showing definite depression; and taken just prior to injection of another grain of morphine sulphate. *E* = Increase in knee jerk immediately following the intravenous injection of ¼ mgm. strychnine sulphate at *2* given 1 hour, 20 minutes after the last injection of morphine. *F* = Record taken 27 minutes after the administration of strychnine. During *E* reflex action below transection almost nil. During *F* brisk reflexes from tail and feet.

Fig. 5. Showing the character of the human knee jerk.

A = Before administration of an 80 per cent C_2H_4 — 20 per cent O_2 mixture. *B* = During the state of unconsciousness arising from the inhalation of the C_2H_4 — O_2 mixture. *C* = During the period of recovery following light anesthesia with C_2H_4 — O_2.

(See fig. 1.) After this preliminary period morphine sulphate was injected subcutaneously. This was followed by a gradual depression with ultimate disappearance of the knee jerk. The time for this to occur was from two to three hours, and during this period eight hundred to twelve hundred knee jerks were recorded.

2. *The effect of morphine sulphate on the cord following transection in the lumbo-thoracic region (acute transection).* In this series of animals the cord

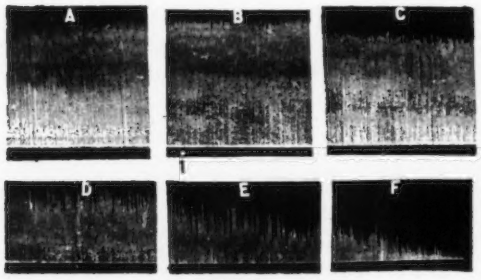
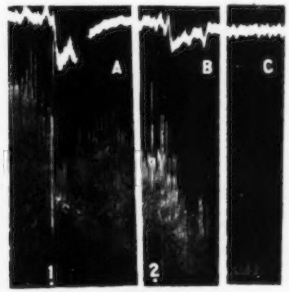


FIG 1



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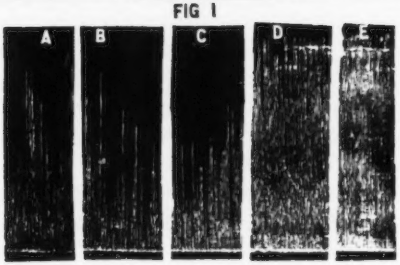

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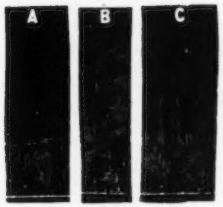

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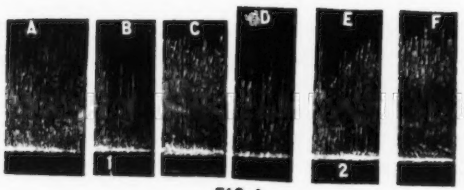

FIG 4

was cut at about the twelfth thoracic vertebra and sufficient time was allowed for the return of the knee jerk before starting any other procedures. These experiments were also done under barbital anesthesia (200 to 250 mgm. per K) during which time morphine sulphate was injected subcutaneously. The results were essentially the same (fig. 2). Obviously spinal and surgical shock rendered the animal already deeply anesthetized unsuitable for prolonged experimentation. A third series of experiments

was therefore carried out on animals with chronic transections of the spinal cord, at various intervals during the recovery of the animals from the anesthetic and from the spinal shock resulting from the transection. Such preparations allowed us to study the effects of morphine on the spinal cord in the *unanesthetized animal.*

3. *The effect of morphine sulphate on the lumbo sacral cord of an unanesthetized animal.* All of these animals were operated under aseptic technique using ether as an anesthetic. In all dogs the knee jerk returned the same day of the transection. The effect of morphine was then studied at various intervals during recovery from spinal shock. In one case the transection was of two years standing. In the dogs of very recent transection (one day), it was possible to obtain a very regular brisk jerk and the results very closely simulated those obtained from a barbitalized dog with intact cord. With recovery from spinal shock, the cord became more irritable, and more influenced by incoming stimuli (the exteroceptors, interoceptors and proprioceptors). As a result the recorded jerk became more irregular. By giving morphine sulphate in doses of one-quarter to 18 grains, the jerk became more regular and possibly very slightly increased in height, lasting at most for five minutes. But after this primary pseudo stimulation there was a gradual and long lasting depression of the jerk (figs. 3 and 4). The results obtained in a dog after complete recovery from spinal shock (after two years) needs special mention. The lower cord was so irritable that it was not possible to register knee jerks, since a blow to the patellar tendon would elicit a series of spontaneous clonic movements. After morphine sulphate ($\frac{1}{2}$ gr.) was injected it was not only possible to register knee jerks, but the results were essentially like those obtained from animals which had partially recovered from shock.

Discussion. *The pseudo-excitatory effect of morphine sulphate on the spinal cord.* There is no doubt as to the fact that morphine in moderate doses ($\frac{1}{4}$ to 18 gr.) causes an increase in the height of the knee jerk in the unanesthetized dog, but the question arises as to whether this rise shall be interpreted as a real stimulation of the cord, or as a pseudo stimulation. The evidence indicates that the morphine not only releases the cord from control of the higher centers, but also decreases the effect which inhibitory impulses from the peripheral structures have upon it. Before the injection of the morphine, movements of the legs occurred either spontaneously or could be induced by pinching the pads of the feet, tip of the tail, or by irritating the mucous membrane of the rectum or vagina. The effect of such stimulation either inhibits the knee jerk entirely, or causes a powerful flexion reflex of the leg from which the knee jerk is being taken and interferes mechanically with the registration of the kick. After the injection of morphine, such stimulations are ineffective. During this analgesic state the knee jerk center is quite isolated from the influence of

such exteroceptive, interoceptive and proprioceptive impulses which are acting normally or are induced artificially. As a result, the limbs are more flaccid and various cord reflexes are obtained with greater difficulty. The more primitive knee jerk is effected least and appears to be more marked and regular since its center is influenced less by afferent inhibitory impulses or the mechanical conditions (flexion of the leg) which such impulses bring about. The increase in the height of the kick, according to these facts and their interpretation, denotes not an increased excitability of the cord but a release of the center responsible for the knee jerk from the disturbing influences of a variety of afferent impulses or the mechanical change in the leg which they affect. We are dealing not with a real but with a pseudo-stimulating action of this drug.

The removal of the inhibitory influences which affect the spinal cord (as measured by the knee jerk). First it might be well to mention some of the direct evidence that the knee jerk under normal conditions is constantly being influenced by impulses from various regions.

The removal of inhibitory influences by anesthetics. It is commonly accepted that the first effect of anesthesia is removal of cortical inhibitions, and this is well exemplified in the effect of an anesthetic upon the knee jerk during the induction of anesthesia. This was done in the dog using ether, paraldehyd, and Na barbital, and in each case the primary effect was an exaggeration followed by depression of the jerk as the state of the anesthesia deepened.

This experiment was repeated on man, using ethylene and oxygen (80 per cent ethylene and 40 per cent oxygen), since it is relatively harmless and can be repeated often without danger. The results were essentially the same, although the procedure was never carried to a stage beyond light anesthesia. Suffice it to say that in two subjects anesthetized to the point of unconsciousness with an 80 per cent ethylene and 20 per cent oxygen mixture, the amplitude of the knee jerk was at least doubled during the period of anesthesia, returning to a normal height on the withdrawal of the anesthetic (fig. 5).

The significance of these results, as related to the action of morphine, can only be appreciated when one observes the striking resemblance between the action of morphine and the action of various anesthetics.

If, for instance, one works with an animal under light anesthesia, it will be found usually that the jerk is irregular, if present at all. By deepening the anesthesia, the jerk returns and becomes markedly increased and of uniform amplitude. If the anesthesia is pushed still further the jerk disappears and if at this time (in the case of volatile anesthetics) the anesthetic is removed, the character of the jerk changes in the reverse order, i.e., the jerk returns to a high level and is regular and then becomes depressed and irregular. In the case of morphine, we can carry the experi-

ment to the point of disappearance of the jerk as a result of excessive depression, but we have not as yet recorded the return, but observation of the animals on the succeeding day indicates that the results would be essentially the same. It is understood that the effect of morphine is of much longer duration than a volatile anesthetic.

A pseudo-depression of the cord by means of the injection of strychnine. It is commonly accepted that strychnine acts as a stimulant to the spinal cord, and this result has also been our finding. But under certain circumstances, we obtained a depression of the knee jerk by the injection of small doses of strychnine sulphate (1 mgm.).

If an unanesthetized dog with a chronic transection (12th thoracic) is used, in which sufficient time has been allowed for recovery from spinal and surgical shock (2 weeks), it was found that the injection of a small dose of strychnine sulphate not only caused a depression of the knee jerk, but it also caused an increase in the general excitability of the animals. There were increased spontaneous movements; and other reflexes such as the extensor thrust, crossed extension, flexion reflex, and tail reflex were much more easily elicited. We fully realize that the general increase in tone of the lower extremities of such an animal on the verge of mild strychnine convulsions may mechanically interfere with the registration of the knee jerk, especially if the electro-magnetic hammer strikes the patellar tendon when the leg is in a marked state of flexion. On the basis of the record alone, the knee jerk appears to be depressed, although it is, in fact, actually exaggerated. Mechanical factors simply prevent its full registration.

On the other hand, if the animal was deeply anesthetized a small but adequate dose of strychnine would almost invariably cause an accentuation of the jerk.

SUMMARY AND CONCLUSIONS

The effect of moderate doses ($\frac{1}{4}$ to 18 gr.) of morphine sulphate on the excitability of the spinal cord, as illustrated by a quantitative study of the knee jerk, was determined in the intact, but anesthetized dogs, in dogs whose spinal cords were transected, but still anesthetized (with Na barbital), and in unanesthetized dogs at various time intervals while recovering from spinal shock. Our results are as follows:

1. In an animal with intact cord but deeply anesthetized, morphine in any effective dose ($\frac{1}{8}$ to $\frac{1}{4}$ gr.) depresses the spinal cord as judged by the amplitude of the knee jerk.

2. In the unanesthetized animal whose cord has been transected in the lumbo-thoracic region (acute experiment), morphine sulphate in the same dose also depresses the spinal cord as judged by the amplitude of the knee jerk.

3. In unanesthetized dogs recovering from spinal shock, morphine sulphate in moderate doses ($\frac{1}{4}$ to 18 gr.) likewise effects a depression of the spinal cord as judged by the disappearance of spontaneous reflex movements posterior to the transection and the difficulty of eliciting reflexes posterior to the section by various afferent stimuli. The increase in amplitude of the knee jerk under such conditions is interpreted as due to a release of the knee jerk centers from the inhibitory control of impulses from a variety of organs and tissues and absence of mechanical antagonistic restraint due to diminution in tone of muscles antagonistic to the quadriceps extensor.

We therefore conclude that the primary effect of morphine sulphate in the doses used is a depression of the reflex excitability of the cord and the marked briskness of the knee jerk observed temporarily following injection of morphine sulphate in the intact animal is due parly to an isolation of the knee jerk centers from the influence of impulses which normally act on it and partly to the diminution in tone of the antagonistic muscles which tend to reduce the amplitude of the kick.

BIBLIOGRAPHY

(1) Tatum and Collins. 1926. Journ. Pharm. Exper. Therap., xxvii, 237.
(2) Sloan, J. H. 1926. This Journal, lxxix, 100.
(3) Johnson, C. A. 1927. This Journal, lxxxii, 75.

STUDIES ON THE KNEE JERK

III. The Effect of Raised Intrapulmonic Pressure upon the Knee Jerk, Arterial Blood Pressure and the State of Consciousness

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Received for publication September 21, 1927

It seems to be rather common knowledge that the human being can be rendered giddy or more or less unconscious by sufficient pressure upon the chest—the pressure being applied during full inspiration. This procedure has been practiced, and still is, as a common trick in fraternity circles and other social gatherings. The practice was first called to our attention by medical students who inquired concerning the physiological mechanism involved. No plausible or satisfactory answer could be offered at the time and the matter was forgotten. In the course of some studies on the influence of raised intrapulmonic pressure on the knee jerk of dogs the casual question put to us years ago by medical students seemed worthy of solution because of its theoretical and practical aspects.

The fact has probably been known for a long time that raising and maintaining the intrapulmonic pressure (either by pressure externally upon the chest walls when the lungs are maintained in a state of inspiration, or by inflating the lungs through the trachea and then clamping the latter) causes a marked fall in blood pressure. This procedure is but a modification of the experiment of Valsalva (1666–1723) known to every physiologist. At the time we first performed it in its modified form, we were neither interested in the subjective symptoms which had been described by this early observer, nor particularly in the effects of the procedure on the general arterial blood pressure which we happened to be taking at the time. Our immediate interest was in the effects of raised intrapulmonic pressure on the reflex excitability of the cord, using the amplitude of the patellar jerk as a criterion of cord excitability, for it was thought possible that the mechanical stimulation of the sensory fibers of the cardiac and pulmonary vagus might reflexly affect the knee jerk center.

Since the knee jerk center was profoundly affected by this maneuver, coincidently with a more or less precipitate drop in blood pressure, it seemed desirable to determine the relation, if any, between the drop in

[1] Aided by a grant from the Frank Billings Medical Club, Chicago.

general arterial blood pressure and the feeble activity of the knee jerk. In the course of the investigation we also obtained data throwing light on the probable mechanism responsible for the giddiness and unconsciousness resulting from powerful compression of the chest during deep forced inspiration. The results obtained on these two questions form the body of this report.

METHODS. *1. Experiments on animals.* Dogs were used in various states of anesthesia and under various anesthetics (paraldehyde, ether and sodium barbital). In some animals the cord was left intact. Other dogs had suffered a transection of the cord previous to the experiment. Blood pressure records were taken from the carotid artery. In some of the animals a double vagotomy was performed in the cervical region. In others the cardiac inhibitory nerve endings were paralyzed with atropine. Suitable doses of strychnine were injected to produce mild convulsions. The effect upon the knee jerk of stimulation of both the central and the peripheral ends of the vagi were noted. Continuous records of the knee jerk were taken by means of the apparatus described by one of us (C. A. J.), in the first article of this series (1). Raised intrapulmonic pressure was usually effected by inflating the lungs through a tracheal tube provided with rubber tubing and then clamping the latter.

2. Experiments on man. By means of the artificial respiration machine devised by Dr. K. A. Fries, it was possible to apply varying degrees of pressure to the chest during deep inspiration while the subject was in a reclining position. The systolic blood pressure was measured with a sphygmomanometer (Tycos) before the external pressure was applied to the thorax, at the time of greatest drop in blood pressure, during the period immediately after the release of the external pressure and raised intrapulmonic pressure (to obtain the after-fling), and shortly after this to obtain the return to normal.

RESULTS. *1. Effects on the blood pressure.* In all cases after applying the various procedures mentioned above, there was a sudden fall in the carotid pressure in the case of the dog and the brachial pressure in the case of man. The low pressure usually could be maintained as long as the intrapulmonic pressure was maintained.

a. In the dog the carotid pressure could be dropped to zero by marked distention which was maintained by occlusion of the trachea. When the pressure was released, using dogs under light anesthesia, there was a rapid rise above normal which amounted in some cases to as much as 110 mm. Hg. The extent of the after-fling depends directly on the length of time that the raised intrapulmonic pressure was maintained and the degree of distention produced (fig. 1).

b. The results on man were essentially the same. The experimental maneuver used on man and described under Methods was designed to

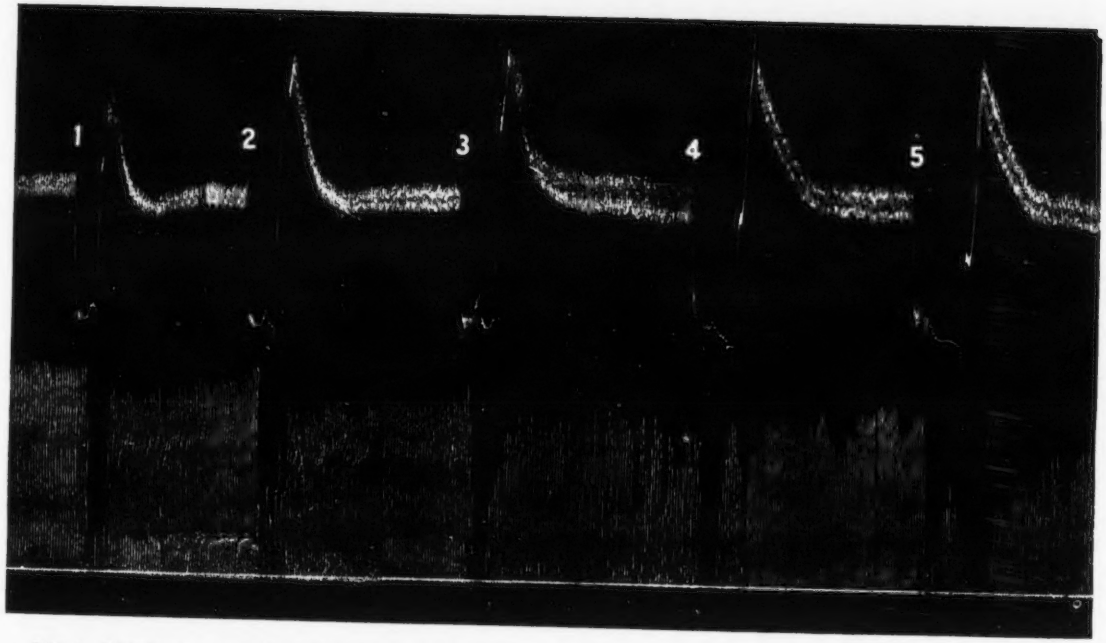

Fig. 1. Continuous record of the carotid blood pressure and knee jerk in a 14 kgm. barbitalised dog (4½ grams) showing the effect on both of suddenly raising the intrapulmonic pressure and maintaining it at *1* for 45 seconds, and at *2* for 1 minute, at *3* for 1 minute and 15 seconds, at *4* for 1 minute and 30 seconds, and at *5* for 1 minute and 45 seconds.

simulate the feat performed at social gatherings at which the demonstrator, standing behind the volunteer "victim," encircles the latter's chest with his arms, lifts him clear from the floor, and holds him there until as a result of either asphyxia or unconsciousness or both, involuntary respiration is resumed by the subject experimented on.

By using moderate pressure it was possible to cause a drop in brachial pressure of 40 to 60 mm. Hg below normal (table 1).

When the external pressure was released there invariably occurred an after-fling of from 20 to 30 mm. Hg above normal. Concomitantly with this after fling there was a pounding in the head and if the procedure was repeated several times there remained an after-distress which lasted for two to three hours, manifesting itself in headache, nausea, malaise and slight ataxia.

TABLE 1

Illustrating the effect of applying pressure to the chest during sustained deep inspiration on the brachial pressure in man

SUBJECT	NORMAL SYSTOLIC	SYSTOLIC DURING COMPRESSION	AFTER EFFECT SYSTOLIC	DROP IN SYSTOLIC	TOTAL CHANGE
E	114	72	140	42	68
E	112	72	142	40	70
L	140	90	162	50	72
L	140	94	164	46	70
J	128	100	140	28	40
J	128	90	140	38	50

2. Effects upon the knee jerk in partially anesthetised dogs. The effect on the knee jerk from any of the procedures mentioned above is dependent upon the stage of anesthesia.

If the anesthesia was light, the knee jerk was usually irregular, if present at all. The reasons for this have been discussed in a previous paper (2). By lowering the blood pressure and thus inducing a slight amount of central shock by cerebral anemia the knee jerk mechanism is released from central control and peripheral inhibitory (?) impulses. The mechanical factors which tend to modify the knee jerk are also improved by relaxation of the muscles antagonistic to the quadriceps femoris (fig. 2). This allows for a higher and more regular jerk which is maintained until the temporary shock wears off with the return of the blood pressure to its normal level, when the knee jerk returns to its former state. It is further possible in a suitable preparation to induce such a great amount of shock that the knee jerk, at first increased, is then secondarily diminished or even abolished as a result of long lasting anemia of the brain and cord because of excessive and long maintained raised or oft repeated intrapulmonic pressure (fig. 2).

The precipitate drop in arterial pressure is due essentially to an almost

complete cessation of circulation through the lungs when the intrapulmonic pressure is raised. The blood being thus prevented mechanically from reaching the left ventricle, an extensive venous congestion ensues. Arterial circulation through the brain and cord and all peripheral structures ceases. The sudden anemia particularly, together with the asphyxia, leads to prompt central shock (brain and cord). Central control over the knee jerk succumbs first and the knee jerk center thus isolated from the

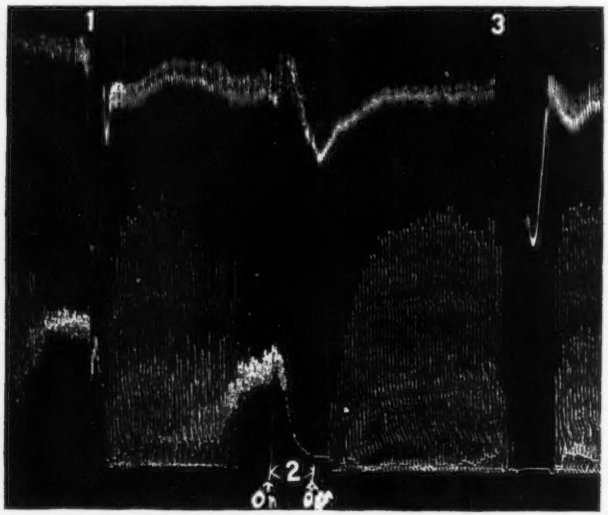

Fig. 2. Tracing of the carotid blood pressure and knee jerk from a dog under *light* paraldehyd anesthesia (1.5 cc. per K) showing at: *1*, The pseudo excitatory effect on the knee jerk center of raising the intrapulmonic pressure. The tone of the leg drops immediately; and the knee jerk becomes more regular in character and higher in amplitude. As the animal recovers from the after effects of the cord anemia, the tone of the leg increases and the knee jerks become lower in amplitude. *2*, Similar pseudo excitatory effection the knee jerk center as at *1* brought about by etherization at *2*. *3*, As the result of previous etherization of the dog already under paraldehyd, raised intrapulmonic pressure effects immediate *depression* of the knee jerk center itself with abolition of knee jerks.

central and peripheral impulses, functions unhampered and, as a result, the individual contractions of the quadriceps are regular and higher. The knee jerk center itself, however, soon succumbs to the anemia if the intrapulmonic pressure is maintained. As a result the patellar jerk is more or less promptly abolished. On releasing the intrapulmonic pressure the recovery of the center from anemia and asphyxia is slow and protracted.

It may take fifteen minutes after reëstablishment of the circulation through the cord before the center has even partially recovered.

Additional evidence can be offered in support of the view given above, that the progressive failure of the knee jerk during raised intrapulmonic pressure is due to interference with the circulation through the brain and cord.

Raising the intrapericardial pressure in a doubly vagotomised dog likewise results in an immediate and profound drop in arterial blood pressure. Coincidently the knee jerk is abolished. On restoring the arterial pressure to the normal level by a release of the raised intrapericardial pressure, the knee jerk again returns. (It should be understood that these animals were under moderately deep anesthesia. The significance of the state of the anesthesia will be considered later.) If in such a preparation one clamps the thoracic aorta, the general arterial blood pressure as measured from the carotid artery, rises appreciably. But circulation through the lumbar cord and the knee jerk center obviously ceases. With the cessation of circulation through the cord the knee jerk is abolished but returns again following removal of the occlusion of the thoracic aorta.

During moderately deep anesthesia, the jerk is usually regular and high for reasons cited in a previous paper of this series (2). By applying the procedures above mentioned there is an immediate fall in the jerk with an ultimate disappearance which returns upon release of the external pressure. If these procedures are repeated several times, there comes a time when each successive repetition does more damage. Some irreparable damage is done for the knee jerk does not return to normal and there is a possible danger of killing the animal even by moderate inflations. (See fig. 1.)

The effects of the above procedures upon the knee jerk after sectioning the vagi. Early in this work it was thought that the sensory fibers in the vagi might play an important rôle in the effect upon the knee jerk as a result of mechanical stimulation during the inflation of the lungs, particularly since apnea vagi is so strikingly in evidence during the period of inflation. The reasons for this view are: 1. The fact that stimulation with a faradic current of the central end of the vagus causes a marked diminution in the height of the knee jerk (under moderate anesthesia) (fig. 3); 2, under moderate anesthesia, raised intrapulmonic pressure caused almost *immediately* a visible depression of the knee jerk.

The results are, as a whole, rather inconclusive but under certain circumstances we were able to show some effect (fig. 4). In the intact animal, under moderately light anesthesia, section of the vagi increases the latent period of the effect of raised intrapulmonic pressure upon the knee jerk. That is, the knee jerk is affected much later after the intrapulmonic pressure has been raised than prior to the double vagotomy. The uncertain results of double vagotomy in a given animal can probably be explained in the

following manner: In the deeply anesthetised and double vagotomised animal sectioning the vagi can presumably produce very little effect, since the nervous mechanisms which can influence the knee jerk center are already greatly depressed by the anesthetic.

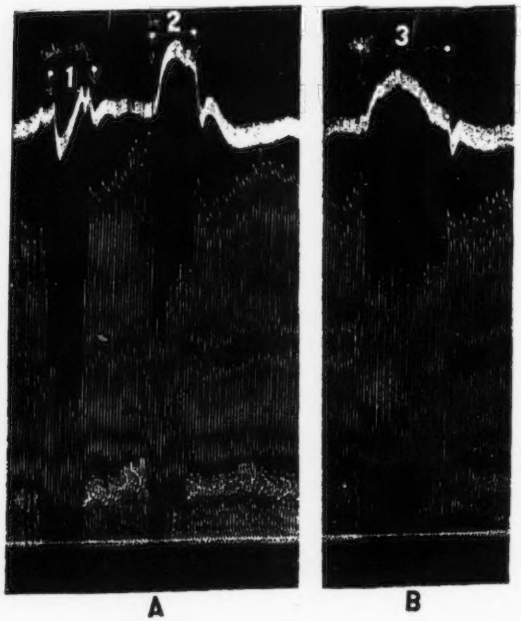

Fig. 3

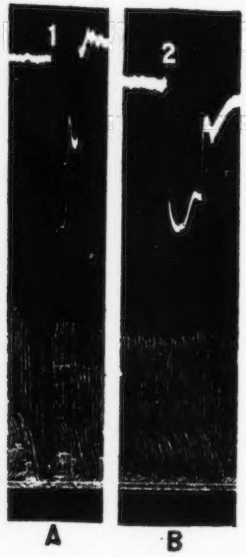

Fig. 4

Fig. 3. Showing the effect in a 16 kgm. dog (4½ grams Barbital Na) of stimulation of the central end of the vagus on the amplitude of the knee jerk. The nerve was stimulated with a tetanising current in A and B between the dots at *1*, *2*, and *3*. Note the *immediate* diminution in the amplitude of the jerk accompanied in one instance by a fall and two instances by an elevation of the general arterial blood pressure.

Fig. 4. Carotid blood pressure and knee jerk records of a dog in moderate barbital anesthesia showing: A *1*, The immediate effect on the knee jerk of raising the intrapulmonic pressure and at B *2*, the absence of any visible effect after double vagotomy even though the raised intrapulmonic pressure was maintained twice as long.

In a few animals whose cords had been transected at the beginning of the experiment, raised intrapulmonic pressure with or without double vagotomy caused a diminution of the knee jerk coincident with, or following shortly after the precipitate drop in the general arterial blood pressure.

The effect of raised intrapulmonic pressure upon an animal in mild strychnine convulsions. On the basis of the various considerations expressed in this and the previous article of this series (2), it seemed possible to release the knee jerk center of the normal lightly anesthetised, or deeply anesthetised and strychninised animal from the various influences of central and peripheral origin which act in such a manner that the registration of a kick of uniform height and moderate amplitude is impossible.

A control period was taken in a given anesthetised animal, and then a suitable dose of strychnine was injected (2 to 4 mgm.) to produce convulsions.

The injection was followed by a marked increase of the knee jerk and then convulsions. During the convulsive state, it obviously was impossible to register the knee jerks, since the extreme spasticity of the leg used for registration prevented more or less any registration. But, after raising the intrapulmonic pressure for a short time, the convulsions disappeared, the animal became quiet and it was again possible to register high knee jerks. After a time convulsions would reappear but it was only necessary to repeat the above procedure to render the animal quiet. In the normal but mildly strychninized and lightly anesthetized animal whose hind limbs showed more or less spasticity and spontaneous movements with no appreciable knee jerks, raised intrapulmonic pressure was followed by a return of powerful knee jerks. In a normal but lightly anesthetized animal etherization also caused a marked increase in the amplitude of the knee jerk (pseudo-excitation) (fig. 2).

The appearance of the knee jerk, under such experimental conditions in a pseudo exaggerated form, cannot be attributed to an asphyxial state of the cord resulting from the suspension of normal respiratory exchange during the raised intrapulmonic pressure; for 1, the greatest increase of the jerk came usually after the release of the raised intrapulmonic pressure; 2, extreme aspiration of air from the lungs followed by clamp on the tracheal tube was immediately followed by marked asphyxial breathing, but no increase in the height and regularity of the knee jerk response.

Discussion. *1. Blood pressure effects.* It is probably understood from what has been already said that raising the intrapulmonic pressure by any of the methods described not only prevents circulation through the lungs but prevents proper filling of the heart because of the direct pressure of the distended lungs against its walls.

The extent of the after response (after-fling or compensatory rise in blood pressure) is probably dependent upon several factors. Its extent, as previously mentioned, is dependent upon the degree and length of time of compression. It is also dependent upon the state of the anesthesia, and the degree of shock.

During moderate or deep anesthesia and various degrees of shock one

could hardly expect the vasomotor mechanism to react significantly, or at all, as a result of asphyxia produced by the raised and maintained intrapulmonic pressure. Using our own experiments as a basis for discussion, one might conclude that the after-fling observed in man and dog, under light anesthesia, is the response of the vasomotor center to the suddenly decreased blood pressure, and particularly the asphyxia resulting therefrom.

The interpretation given is in harmony with the findings of Cushing (3) on raised intracranial pressure, and of Stewart, Guthrie, Burns and Pike (4) in the condition of cerebral anemia.

2. Effects upon the knee jerk. On the basis of this work there appear to be at least three outstanding factors which determine the extent of the knee jerk: a, the state of the knee jerk center; b, the state of the spinal cord, and c, the mechanical factor as a result of contraction or relaxation of the muscles antagonistic to the quadriceps femoris.

a. *The state of the spinal cord.* As indicated in a previous paper of this series (2), the knee jerk is constantly being influenced by afferent impulses from various portions of the body (i.e., exteroceptors, interoceptors, proprioceptors and impulses from the higher centers). Therefore, if the nervous system has been greatly depressed by anesthesia or shock to the extent that these afferents play no rôle in modifying the patellar jerk, a further depression by cessation of circulation through the spinal cord will abolish the jerk.

Conversely, if no anesthesia or light anesthesia is used, induction of shock by this method isolates the knee jerk center from inhibitory afferents and the jerks are higher and more regular.

b. *The state of the knee jerk center and cord.* Using the knee jerk as a criterion of cord excitability it was pointed out in a previous paper of this series (2), that the spinal cord could be depressed by moderate doses of morphine sulphate and hypnotics and could be excited by suitable doses of strychnine sulphate.

c. *The mechanical factor influencing the knee jerk.* There is a purely mechanical factor in the action of the muscles antagonistic to the quadriceps which modify the extent of the jerk. Relaxation of these muscles by central shock or anesthesia permits, in a mechanical way, of a more pronounced extension of the leg from the same degree of contraction of the quadriceps (fig. 2).

On the basis of the observations on dog and man recorded in this communication, we are in a position to condemn the practice of rendering ready volunteers unconscious by compression of the chest during a forced and maintained inspiration. If in the supine position the systolic pressure can be more or less suddenly dropped 40 to 72 mm. of mercury, such compression causes most probably an even greater drop in pressure when the person

is in an upright position, as is usually the case. The giddiness occasioned in the course of the compression results from the impairment of the arterial circulation through the brain, and if in a given individual the compression is so marked and prolonged that the left heart receives very little or no blood, complete unconsciousness (anesthesia) results because of a complete absence of cerebral circulation. The general passive congestion is, to say the least, not beneficial or entirely innocuous. But what is more deleterious is the sudden change in blood pressure from a very low point to one exceeding the normal some 30 mm. or more (man). The more or less sudden and pronounced after-fling may easily lead to rupture of arterioles especially if the latter are already weakened by disease. The sensation of fulness in the head, headache, nausèa, ataxia and vertigo, which are experienced immediately on release of the compression and which may last for hours, are cerebral disturbances resulting from mild injury to the nervous tissues. The experiment is harmful when performed on healthy individuals; it may prove disastrous when performed on an individual having even slight myocardial or vascular lesions.

Parenthetically, we express our conviction that any form of artificial respiration involving compression of the chest (manual or instrumental) is to be preferred to most devices which deliver oxygen or air into the lungs under positive pressure. Aside from the danger of actual rupture of the lung parenchyma by the latter devices, the positive pressure into the lungs, if not most carefully controlled, may induce an acute emphysema, or at least interfere markedly with circulation through the lungs. Since an increase in the intrapulmonic pressure so easily effects a drop in blood pressure, since it interferes with the proper filling of the heart, any artificial respiration machine which forces air into the lungs under positive pressure interferes with circulation through the lungs at each blast of the machine, unless the amount of air is admitted gradually and in an amount not exceeding that normally inspired.

Usually this interference with the flow of blood through the lungs occurs at a time naturally when the general arterial pressure is already dangerously low. Such devices or manipulations which fill the lungs with air as a result of return of the thoracic cage to its normal position due to its own resilience following compression are therefore to be preferred, particularly if the artificial respiration is to be carried on for some time.

CONCLUSIONS

1. In the dog raised and maintained intrapulmonic pressure leads to a *sharp drop in the general arterial blood pressure* accompanied by a *diminution in the knee jerk* if the animal is shocked or is under deep anesthesia; or to a pseudo-augmentation of the jerk if the animal is under light anesthesia. The effects of this procedure on the knee jerk are due, for the most part, in

either case to changes in circulation through the brain and cord (anemia and asphyxial depression).

2. Some evidence is presented, however, supporting the view that the diminution of the knee jerk, observed during raised intrapulmonic pressure, may be due to reflex inhibitory effect on the knee jerk center as a result of the mechanical stimulation of the sensory fibers of the vagus.

3. The suspended circulation through the cord, during raised and maintained intrapulmonic pressure, results from marked interference with the filling of the heart. The blood, during raised and maintained intrapulmonic pressure, is prevented from reaching the left heart.

4. The giddiness or actual unconsciousness induced in man following powerful compression of the chest, after and during deep maintained inspiration is attributed essentially to the drop in general arterial blood pressure and the cerebral anemia resulting therefrom. The compensatory after-fling is responsible for the subjective symptoms of nausea, fulness in the head, and general discomfort which are experienced immediately after release of the raised intrapulmonic pressure.

BIBLIOGRAPHY

(1) JOHNSON, C. A. 1927. This Journal, lxxxii, 75.
(2) LUCKHARDT AND JOHNSON. 1928. This Journal, lxxxiii, 634.
(3) CUSHING. 1901. Johns Hopkins Bull., xii, 290; Amer. Journ. Med. Sci., N. S., 1902, cxxiv, 375.
(4) STEWART, GUTHRIE, BURNS AND PIKE. 1906. Journ. Exper. Med., viii, 289.

STUDIES ON THE KNEE JERK

IV. The Effect of Moderate Doses of Morphine Sulphate on the Knee Jerk of the Cat

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Received for publication October 10, 1927

In a previous paper of this series, we (Luckhardt and Johnson, 1927) described the effect of moderate doses of morphine sulphate on the knee jerk mechanism of the dog. Since that time this particular problem has been extended to cats.

The prevalent view is that cats exhibit excitatory effects chiefly as a result of the injection of moderate doses of morphine sulphate. The question arose as to whether the excitation observed was a real or pseudo-excitation manifesting itself as a result of removal of inhibitions and allowing the animal to act in a more or less incoördinate manner.

The true or false excitation interested us only insofar as it was related to the knee jerk; and it is this particular phase of the problem which we wish to report on briefly at this time.

Methods. By means of a special knee jerk apparatus, described by one of us in a previous paper of this series (Johnson, 1927), it is possible to deliver constant blows to the patellar tendon at a definite interval and quantitatively record the responses over a long period of time.

Cats were used whose cords were either intact or had been transected just prior to the beginning of the experiment. The effect of moderate doses of morphine sulphate ($\frac{1}{4}$ to 2 grains) on the knee jerk center was recorded from animals in various stages of anesthesia and under several different anesthetics (barbital -Na, ether and paraldehyde). In several cases small doses of strychnine sulphate ($\frac{1}{2}$ to 2 mgm.) were injected during the course of an experiment in order to contrast its effect with that of the previously injected morphine.

A typical experiment consisted in: 1, placing the animal in the desired stage of anesthesia; 2, arranging the animal to record knee jerks as described in a previous paper; 3, taking a control period of from two to three hundred knee jerks (taking from $\frac{1}{2}$ to $\frac{3}{4}$ hour); 4, injecting a suitable dose of morphine

[1] This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

sulphate ($\frac{1}{4}$ to 2 grains); 5, taking records until a maximum depression occurred, and 6, injecting a small dose of strychnine sulphate ($\frac{1}{2}$ to 2 mgm.). Such an experiment lasted from two to six hours during which time one thousand to three thousand knee jerks were recorded.

RESULTS. Although in no case did we use any animals totally free from an anesthetic, the results were essentially the same as those found in the dog and recorded in a previous paper of this series (1). The results on the cat are briefly summarised as follows:

In the moderately anesthetised cat, effective doses of morphine sulphate ($\frac{1}{4}$ to 2 grains) caused a quantitative drop in the knee jerk (fig. 1) which lasted in many cases for three to four hours followed by a gradual period of

Fig. 1. Showing continuous record of the patellar jerk of a cat under barbital-Na anesthesia (1 gram injected intraperitoneally).

A = A 30 minute normal record of this type was obtained. *B* = at *1*, $\frac{1}{2}$ grain morphine sulphate was injected subcutaneously. Respiration, 15 per minute. *C* = Record taken 10 minutes later. Respiration, 8 per minute. *D* = Record taken 25 minutes after injection. Respiration, 5 per minute. *E* = Record taken 50 minutes after the injection of morphine. *F* = Record taken 80 minutes after injection of morphine. Knee jerk still abolished. At *1* injection of 2 mgm. strychnine sulphate subcutaneously. *G* = At *1*, taken 10 minutes after strychnine injection. Note particularly the diagrammatic rise in the amplitude of the knee jerk to a height twice that of normal, *A*.

Fig. 2. Continuous record of the knee jerk of a lightly anesthetized cat ($\frac{1}{2}$ gram barbital-Na intraperitoneally) before and after the subcutaneous injection of morphine sulphate.

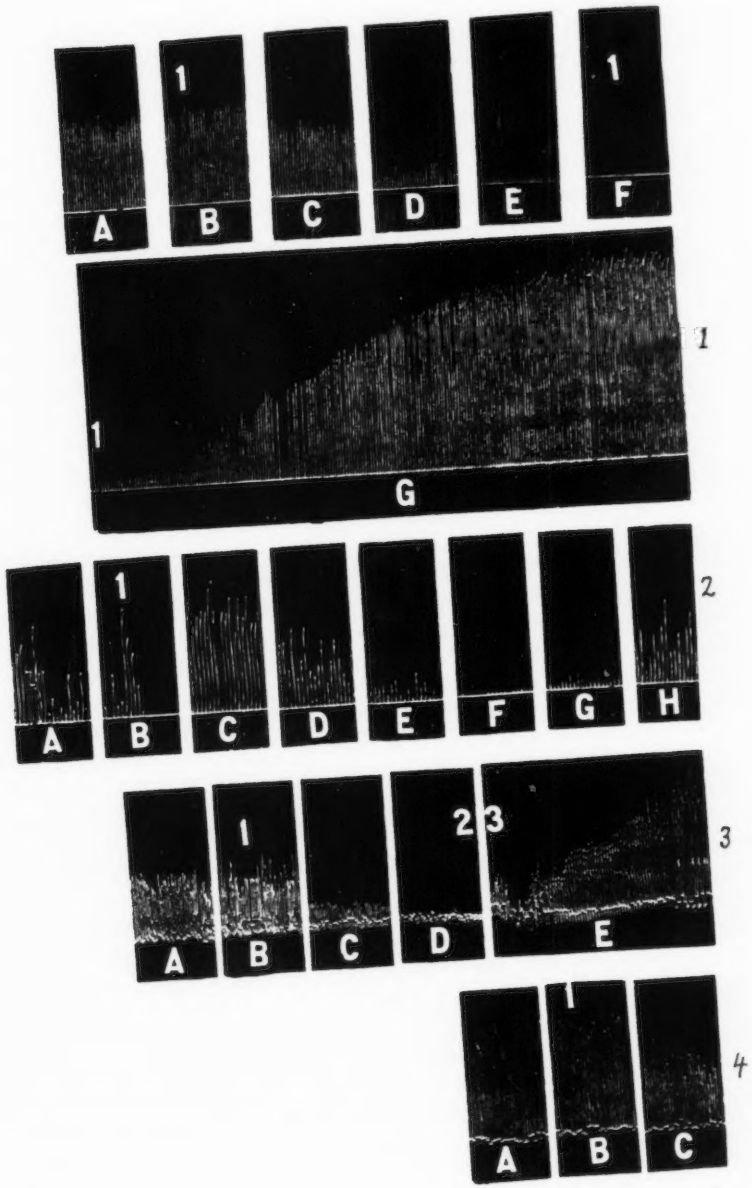
A = Irregular normal knee jerk. During this time animal moved limbs spontaneously. Flexion reflex and crossed extension were easily elicited. *B* = At *1*, $\frac{1}{2}$ grain morphine sulphate was injected subcutaneously. *C* = Record taken 15 minutes after the injection of the morphine shows a knee jerk of greater amplitude and greater regularity (removal of factors discussed in a previous papers). *D* = Record taken 30 minutes after injection of morphine sulphate. *E* and *F*, 40 and 50 minutes respectively after the injection of the morphine sulphate. Knee jerk completely abolished at this time. *G* and *H* = Records taken $3\frac{1}{2}$ and $4\frac{1}{4}$ hours after injection of the morphine and showing recovery from the original depression produced by the drug.

Fig. 3. Continuous knee jerk records of a cat anesthetized with barbital-Na (1 gram given intraperitoneally). Spinal cord transected at the 1st lumbar.

A = Record taken 2 hours after transection of the cord. *B* = 1 grain of morphine sulphate given subcutaneously at *1*. *C* = Extent of the depression of the knee jerk center 30 minutes after injection of the morphine sulphate. *D* = Knee jerk record 1 hour 10 minutes after the morphine sulphate injection. At *2*, 2 mgm. strychnine sulphate given subcutaneously. *E* = At *3*, 12 minutes after injection of the strychnine. Increased excitability as measured by height of the knee jerk, plainly seen.

Fig. 4. Knee jerk record of cat with transected cord taken 24 hours after experiment recorded in figure 3. Animal still under influence of sodium barbital.

A = Record of "normal" knee jerk. *B* = At *1*, injected $\frac{3}{4}$ grain morphine sulphate subcutaneously. *C* = Depression in knee jerk observed 35 minutes after injection of the morphine sulphate.


A
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recovery. If in this period of depression, a small dose of strychnine sulphate ($\frac{1}{2}$ to 2 mgm.) was injected the knee jerk returned to the normal, or even higher than the normal (fig. 1, *G* at *1*).

In the lightly anesthetised cat a slightly different situation exists (fig. 2). Figure 2, *A*, is a representative period of the normal. The knee jerk is irregular and low. Morphine sulphate was given at figure 2, *B* at *1* and it is seen that the jerk becomes temporarily higher in amplitude and more regular, *C*. Following this there is a depression of the jerk and finally after several hours a spontaneous recovery.

In the anesthetized animal whose cord has been transected immediately preceding the beginning of the experiment, the results are essentially the same as in the intact animal. Figure 3 illustrates a typical result in which a small dose of morphine sulphate injected at *B*, *I*, effected a depression of the knee jerk (*C* and *D*). A subsequent dose of strychnine sulphate effected an increase in the knee jerk at *E*.

In most cases our results indicate that the depression, as a result of the injection of morphine, is more transitory in the cat than in the dog. Figure 2 illustrates a typical record in which the animal showed a spontaneous return of the knee jerk three and one-half hours to four and one quarter hours (*G* and *H*) after the injection of the morphine sulphate. We fully realize that this return may be an artifact, since cats show considerable variability in their resistance to anesthetics. In this particular case the depression of the knee jerk undoubtedly was due in part to the Na barbital. If the Na barbital is eliminated the depression of the knee jerk center would not be as great in spite of the morphine injected. Figure 4 shows at *C* a less pronounced depression of the knee jerk under conditions when the animal was only slightly under the influence of Na barbital.

Discussion. Removing inhibitions by any method in man, dog or cat gives the *appearance* of excitation without really being a true excitation. We have in mind the apparent excitatory effect of moderate doses of ethyl alcohol which, according to vast amount of evidence, seems never to excite.

One need only to consider the induction of anesthesia in any of these animals—there is fear, the pupils become dilated, and the movements become incoördinate and exaggerated until finally they cease as the result of depression of the central nervous system. The animal has the appearance of being excited but the motor manifestations may be due only to the loss of inhibitory control. As in the dog we are inclined to the view, on the basis of the evidence presented in this paper, that the primary effect of morphine in the cat is a depression since 1, the knee jerk becomes smaller in amplitude as a result of the injection of this drug coincidently with a marked diminution in the briskness of flexion, crossed extension and tail reflexes; and since 2, strychnine sulphate injected under identical conditions

leads initially to a marked increase in the amplitude of the knee jerks, the jerks in many instances being higher than normal jerks recorded prior to the injection of the morphine.

SUMMARY AND CONCLUSIONS

The effect of moderate doses of morphine sulphate ($\frac{1}{4}$ to 2 grains) on the excitability of the knee jerk center of the cat, as illustrated by a quantitative measurement of knee jerks under controlled conditions, was determined in intact cats and cats with transected cords under various stages of anesthesia and under various anesthetics. Our results are as follows:

1. In the cat (cord intact, but moderately or lightly anesthetized) morphine sulphate in effective doses ($\frac{1}{4}$ grain) depresses the knee jerk, as judged by the amplitude of the response.

2. In cats (cords transected at first lumbar but moderately or lightly anesthetized) morphine in the doses used ($\frac{1}{4}$ to 2 grains) causes a depression of the knee jerk center.

BIBLIOGRAPHY

(1) Luckhardt, A. B. and C. A. Johnson. 1928. This Journal, lxxxiii, 589.
(2) Johnson, C. A. 1927. This Journal, lxxxii, 75.

STUDIES IN THE PHYSIOLOGY OF EXERCISE

I. Exercise and Basal Metabolism in Dogs

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Received for publication October 4, 1927

Under the general head of influence of muscular work on basal metabolism there have been presented as many different results as there are possible answers. Thus some claim an increase, others a decrease, and a third group believes the metabolism remains unchanged.

Benedict (1915) reviews the factors affecting basal metabolism under two main heads, namely, "the active protoplasmic mass" and "stimulus to cellular activity." He places athletes in the class with individuals having a high proportion of active protoplasm and therefore a higher metabolic rate than the average person. Further, he provides for increased basal metabolism by the after-effects of severe muscular work. This latter is presumably a transient stimulus to cellular activity.

The literature is most easily analyzed for our purpose under four main headings as follows:

1. Variations in metabolism supposedly due to a greater or lesser amount of musculature.
2. Variations due to "after-effects" of exercise on the previous day.
3. Variations in and out of training in which "after-effects" are ruled out and there is no reason to assume sufficient changes in the proportion existing between the muscular and non-muscular fractions of the body weight. The variations here would be correlated with a change in general condition sometimes designated "trained" and "untrained."
4. Normal variations in metabolism.

The distinctions here pointed out between after-effects lasting long enough to affect the test of the next day and effects due to a period of training, have not been recognized by all workers and therefore some of the

[1] A grant from the Laura Spelman Rockefeller Memorial to The Young Men's Christian Association College has aided in the conduct of this study.

results presented cannot be accurately judged. If, for example, tests are taken daily and exercise given daily an immediate rise at the beginning of a training period, even though it continued throughout the period, might be interpreted purely as "after-effects"; or if there were at first a rise which disappeared as exercise persisted, the conclusion would be that with better training "after-effects" were of shorter duration. But to establish a certain change as a difference in metabolic level induced by training one would have to find the change coming on gradually as training progressed and then continuing for a reasonable time after cessation of training.

1. Zuntz and Schumberg (1901) reported that during a period of two and one-half months' training (24.75 K. march with 31.5 K. pack daily) the resting metabolism of two subjects, "Herrn P" and "Herrn B", increased 3.4 per cent and 11.2 per cent despite weight losses of 2.85 K. and 1.75 K. respectively. The authors' claim that the two men put on a greater musculature in place of the fat lost is not borne out by the anthropometric data given. In fact, all measurements, including girths of thigh and legs, show either decreases or a stationary condition. A 1 cm. increase in calf girth in "Herrn B" is an exception. All of the tests were taken after a light breakfast which was not completely controlled. Allowing the validity of this criticism, the data on "Herrn B" nevertheless indicate an increased metabolism. It should be added that the experiment was begun in April and ended in July. Benedict and Carpenter (1910) and Benedict and Smith (1915) compare athletes with non-athletes of similar body weight, age, and proportion. According to the earlier work, in a study of many subjects, four who showed distinctly lower metabolic rates turned out to be trained athletes. They say, "These results are entirely at variance with what would naturally be expected from most of the early researches. The fact that the results are so uniformly low with four different subjects cannot be overlooked and yet we wish to state distinctly that the experiments were not planned primarily with the idea of studying athletes and comparing them with a group of non-athletes and hence, before final conclusions are made, we hope to have a series of experiments in which this special problem is studied by itself." In the later work they studied fifteen athletes and compared them in corresponding height-weight groups with thirty-three ordinary individuals reported earlier. In five groups, mostly the heavier weights, athlete metabolism exceeded that of non-athlete by from 8 to 19 per cent, whereas in the remaining five groups, mostly lighter men, the differences ran from 1 to 3 per cent, again favoring the athletes. The grand average for the series shows the athletes about 7 per cent above the non-athletes. They conclude, ". . . . it is hardly to be supposed that the effects of work carried out the day before would so persist for over twelve hours as to account for the larger metabolism noted

in the majority of these athletes. We believe we are therefore justified in concluding that the increased proportion of active protoplasmic tissue present in the trained, hardened athlete is alone sufficient to account for the increase in the metabolism, and that this is not only an absolute increase, but from the nature of the comparison, the metabolism is likewise increased per kilogram of body weight and per square meter of body surface." Later Harris and Benedict (1919) calculated these athletes to average 3.03 per cent above the normal based on their multiple factor prediction standards. But Krogh, quoted by Boothby and Sandiford (1924), found that 102 ordinary individuals published by Boothby and Sandiford averaged 4.8 per cent higher than the same Harris and Benedict standards.

2. On the question of "after-effects" Benedict and Cathcart (1913) show their professional athlete to be regularly 10 to 15 per cent above normal two to four hours after cessation of exercise on a bicycle ergometer. Hill (1926) finds in runners, "after severe or prolonged exercise it may be 80 minutes before the oxygen intake is at its resting value again." Wishart (1927b), from a long series of experiments using the hand ergometer finds, "The after effects of an hour's moderately severe muscular work, quantitatively in raising the basal metabolism and qualitatively in altering the metabolism in the sense of a lower R.Q., is still in evidence on the day following the work (in the present experiments, 21 hours after the work was done). The differences (1 to 2 per cent) are so small, however, as to be entirely obscured in occasional observations by the normal variations in the metabolic rate."

3. The results definitely attributed to "training" in the sense here used are contradictory. Zuntz (1903), reviewing his work with Schumberg (1901), says, "Hier kann man immerhin annehmen dass unter Fettschwund die Muskelmasse zugenommen habe und dass die grössere Arbeit nur indirect, dadurch, dass sie das Wachsen der Muskeln anregte, den Ruhestoffwechsel gesteigert habe." But he says this explanation will not hold in the case of his dog number 6 who ran a resting metabolism of 837 Cal. per sq. m. per 24 hours after almost two years of "Faullenzen," then after four weeks of training in running on a treadmill, ran 1111 Cal. per sq. m. per 24 hours. During the four weeks the dog's weight fell from 29.38 to 27.6 K. He says, "Wir haben hier einen directen Beweis dafür, dass bei andauernd stärkerer arbeit der Ruhestoffweschel zunimmt, dass also die Qualität der Muskeln sich derart ändern muss, dass der Ruheverbrauch grösser wird." In a footnote he adds that the dog was fed 42 grams rice, 17 grams meat powder and 7 grams fat six hours before the test which gave the 1111 Cal. and 150 grams of cane sugar five hours before the test. Not as sensitive to the specific dynamic action of proteins as we are today and reasoning from an experiment on the same dog in which Magnus-Levy fed

him 300 grams rice before a test, he concludes that this 1111 Cal. is amply corrected by reducing it to 988 Cal. thus showing still an 18 per cent increase over the untrained state. Before passing, I must point out what seems to be an error in Zuntz's paper. On page 202 he reports dog 6 with a resting metabolism of 946 Cal. at the beginning of the training period and as 837 after two years of inactivity and low protein diet subsequent to the training period, yet in his discussion on page 206, he uses the lower figure 837 as the before training resting metabolism. If we use the 946 as the pre-training figure and his 988 Cal. (corrected post-training figure), the supposed effect due to exercise is further decreased. Lusk (1915), and Lusk and Dubois (1924) reporting on basal metabolism in dogs, say, "After prolonged confinement in a cage without loss of body weight, a dog may manifest a marked reduction in basal metabolism. Recovery from this condition is achieved through exercise." In the later paper they report a reduction from 20 Cal. in the fall to 16.5 in the spring. A summer in the country was followed by a return to the higher level. In a conversation, Professor Lusk told the author that the exercise here was what dogs would naturally take within the limits of a small yard enclosed by chicken wire bordering on a country road. Further, in the 1924 paper, Lusk reports his own metabolism in November, 1913, after strenuous exercise in the summers of 1910–1913, as 80.9 Cal. per hour and that of April, 1924, after 18 months of comparative inactivity, as 63.4 Cal. per hour. His weight was 78 kilos in both instances. Believing the age factor not responsible he concludes that exercise raises the basal metabolism in man and remarks, "The influence of 'caged life' in reducing the basal metabolism in the dog has a probable counterpart in lack of exercise and indoor confinement in man." Kunde and Nordlund (1927) report the metabolisms of several dogs under varying conditions of physical activity. Their dog II showed a reduction of 16.6 per cent below his previous normal at the end of 22 months of rigorously restricted activity during which time he became "very obese." Nine weeks of exercise at moderate speeds on a treadmill brought his metabolism back to slightly above his normal. They say, "The increased metabolism of the period may be due in part to the development of more muscle tissue but possibly more to the difference in metabolic processes which occur in a muscle made physiologically fit by exercise from one reduced to a state of fatigue by inactivity." In the same paper, dog IX showed, in October, an increase of 30.97 per cent over his spring metabolism. The intervening summer had been spent in daily outdoor exercise, and one is reminded of Lusk's dogs. Recently Schneider and co-workers (1927) reported the basal metabolism of five subjects, one a football player who had been studied in pre-training, training and post-training periods. In three, including the regular athlete, the metabolism dropped from 5 to 6 per cent in training and returned to the pre-training

level in the post-training period. The other two showed no change. Because the athlete in the group shows a metabolism of 9 to 14 per cent above the predicted normal and the other four show figures below the standards Schneider grants that, " . . . . the athlete may normally have a more active metabolism than the non-athlete." "But," he continues, "our results show that it is incorrect to assume therefore that training augments the basal metabolism." The author (Steinhaus, 1926) in a preliminary report presented data on two dogs which indicate that intensive physical activity did not increase basal metabolism.

4. In recent years certain authors, notably Lusk and Dubois (1924), have reported on the constancy of basal metabolism. There have been reports on the constancy between species where the correct surface area measurements are used. (Recently Cowgill and Drabkin (1927) published a new surface area formula which they claim brings the metabolism of female dogs into conformity with Benedict and Harris averages for human females.) There have been reports on intra-species and intra-individual constancy. For the purposes of this paper a few references to normal variations in the same individual are desirable.

Lindhard (1912) finds definite variations in basal metabolism which seem to have an orderly association with the seasons. The data taken from two subjects are not complete but Lindhard believes that light is the stimulating factor since light baths in winter brought on a duplication of the higher runs of the summer months. Benedict (1915) reports normal variations from 3.5 per cent to 31.3 per cent and says, "As a rule the greatest variations were found with subjects studied over the longest periods." Palmer, Means and Gamble (1915) report the metabolism of W.W.P. varying 11 per cent within a half-year. Benedict (1915) in commenting on this finds it necessary to invoke a seasonal factor to account for the difference. Lusk and DuBois (1924) report the metabolism of DuBois as varying within a range of $\pm$ 7.6 per cent over a period of 11 years. We have already mentioned the large variation (over 20 per cent of the higher figure) in Lusk's own metabolism comparing 1913 with 1924 and attributed by them to the variations in the activity programs of the preceding months. Bugbee and Simond (1926) found that their control dog in a castration experiment showed a drop of 35.4 per cent over a period of 16 months. A summer in the country induced no marked change. It must be added that the lower of their figures is still 18 per cent above the average for normal dogs as found by Kunde and Steinhaus (1926). Griffith and co-workers (1926) in a "tentative" report found that the basal metabolism in normal adults showed marked variations which they believe may be correlated with the seasons. Wishart (1927a) finds that the day to day variations in normal persons is such that the minimum differs from the maximum by 30 per cent. He finds that a routine daily program will much reduce the

fluctuation. Three of his four subjects show metabolisms consistently (16 to 21 per cent) below the DuBois standards.

From these observations we must conclude that the popular "plus or minus 15 per cent" rule for comparing individuals with published standards has as its sister rule one allowing fluctuation of 30 per cent in the same individual at various times under conditions all of which may be interpreted as "basal" in the common sense.

METHOD EMPLOYED IN THIS EXPERIMENT. *General plan.* Exercise is a normal function. As such it was early conceived that any change which it might produce could be isolated only under the most rigidly controlled conditions. The above analysis of the literature discloses at every turn a lack of control. To avoid the same difficulties we found it necessary to observe the following rules:

1. Standardize diet and sanitary conditions and reduce excitement to a minimum. Our procedure in this respect is described below.

2. Secure records of each dog in pre-training, training, and post-training periods.

3. While a dog is in training to observe simultaneously other dogs not in training to determine the influence of unknown or uncontrollable factors.

Care of animals. The kennels comprise ten individual metal cages $30'' \times 30'' \times 28''$ in a well lighted and ventilated room containing also three "emptying stalls" provided with running water. The dogs are all males. Three times daily they are turned individually into an "emptying stall" and records are made of defecations. When a dog fails to defecate at three successive times he is given an enema. We have found an "air" enema best. Three to five rubber syringes full of air into the rectum is usually successful.

A new dog, upon introduction to the kennels, is kept apart for several days during which time he is bathed, vermifuged, (for a 20 K. dog 5 cc. CCl_4 and 4 drops oil of chenopodium in water by stomach tube followed after one hour by 10 to 15 grams $MgSO_4$ in 500 cc. warm water) and vocalectomized. With these routine precautions on behalf of cleanliness we have kept our animals entirely free from fleas, intestinal parasites, mange, snuffles, and constipation. Mild attacks of a skin itch readily succumb to a lard-sulphur ointment. Odors and barking have been reduced to a minimum.

The kennel temperature has been kept as constant as heating would permit between 22° and 24°C. In the early part of our work this was not always successful. We have no device for cooling in the summer. The dogs here described were never taken out doors during the entire time covered by these experiments.

Activity. In this paper dogs are designated as "exercised" or as "inactive." They are housed at all times in cages which permit a minimum

activity. When let out into an "emptying stall" or for a test, romping about is discouraged. Since barking following vocalectomy is reduced considerably, we have also reduced this source of inter-stimulation. Under such conditions an animal is called "inactive;" it has been our experience that he will come to lie quietly at the bottom of his cage the greater part of the day, becoming more excitable with the approach of the daily feeding period and "emptying" periods. An "exercised" dog is one who has superimposed upon this regime a controlled and measured amount of running on a motor driven treadmill. This tread requires him to run at the rate of a mile in 12 minutes; (in the exercise indicated in fig. 1 it was 14 minutes) on an approximately 22 per cent grade taking from 120 to 160 steps per minute with each leg. A training period is generally started with three minute runs which are extended until he runs 12 to 18 minutes at a time. The animals are trained to run without being tied to the mill. We

TABLE 1

Standard diets

DOG NUMBER	WEIGHT UPON INTRODUCTION TO KENNEL	HIGHEST WEIGHT ATTAINED ON DIET	GROUND HEART	DRY BREAD CRUMBS	WHOLE MILK	COD LIVER OIL	SAW DUST	TOTAL CAL. (APPROX.)	TOTAL N (BY ANAL.)
	kilos	*kilos*	*grams*	*grams*	*cc.*	*cc.*	*grams*		*grams*
4	16.7	21.0	150	75	190	15	10	873	8.56
5	18.1	23.8	150	75	190	15	10	873	8.56
6	25.0	29.4	150	95	190	15	15	915	8.98
7	20.3	22.5	150	75	190	15	10	873	8.56

have found that a dog when tied to the mill may hang nearly half his weight on a leash fastened to his collar.

On running a mile on a 22 per cent grade the animal raises his body to a height of 353.8 meters. Thus a 20 K. dog performs 7,782 kgm. of work in 12 minutes, in addition to carrying his body weight forward a distance of 1625 meters.

Food. The animals were kept on a moderate protein maintenance diet. By experimentation, the diet was adjusted to hold body weight constant. However, over long periods, all of the dogs, though fully grown at the start, have shown increases in weight. The rations are given in detail in table 1. Each dog was given a small bone each day in addition to the above ration. All changes from this standard diet are indicated in the protocols for the individual dogs. *The food was always fresh and scrupulously free from contamination.*

Technique of the tests. All tests were run on a Sanborn Benedict portable metabolism apparatus of the 1923 type. To this was attached a kymo-

graph marketed by W. E. Collins of Boston as a "Chronokymograph." The dogs were trained and connected to the apparatus essentially after the method described by Kunde (1923). For the calculations the method recommended by Roth (1922) was employed. All tests were made in the forenoon at least eighteen hours after feeding and with but a few exceptions twelve hours after exercise. The room temperature was maintained as constant as possible between 20 and 26 degrees. The dogs were always covered except on days when the temperature exceeded 26 degrees, when it was found that they would often quiet more readily upon being uncovered. The length of the rest was always upwards of thirty minutes. It was found that some dogs would give better runs on short rests while others required a longer time. Record was made of whether or not dogs slept during a test but no evidence was secured that the metabolism is lower in sleep than in the well relaxed waking state. The usual tests for leakage were made. High or irregular runs were verified by check tests. The soda lime was changed every two weeks and CO_2 tests made whenever unusually low runs appeared. With all mechanical factors rigidly observed a low run with slow regular breathing is considered a "good" run. The calculations of calories per square meter per twenty-four hours recorded in the tables were read from the graphic calculating device based on Meeh's formula (see fig. 3) and are accurate to within 0.75 per cent. The newer formula of Cowgill and Drabkin (1927) was not employed since its chief improvement, namely, that of greater accuracy in determination of surface area is not as essential when dogs are compared with themselves and with others of closely similar type and because we wanted to profit by the comparisons which could be made with the work of others based on the older Meeh formula. When surface area is determined by any other device, such as the Cowgill-Drabkin nomograph (1927), the chart in figure 3 may be employed for the remaining calculations.

Results. The data are presented in tables 2 and 3, in figures 1 and 2, and in the protocols of the four dogs. All of the data in the tables represent the weekly averages of all tests conducted during the week indicated by the date of its Sunday. The figures are graphic representations of the data in the tables. The exercise periods are indicated on the graphs and in the protocols.

Protocols of individual dogs. Dog 4, a short-haired male, is short legged with large trunk. He was caged and put on the diet indicated above on March 24, 1925. Successful metabolism tests were made beginning April 5th. On July 2nd, after 14 weeks of inactivity and 66 metabolism tests, his exercise was begun. It being summer this could not be pressed. However, in the 94 days of mild exercise up to October 2nd he ran a total of 332 miles. The period shows an increase in weight reaching a maximum (18.0 K) in the middle of September. Between October

2nd and 31st, the exercise was increased and he was trained to run without being tied to the mill. A total of 252 miles was run in the 29 days and his weight dropped about a kilogram. In the midst of it he was at his best from an athletic standpoint. Immediately following (November 2nd to 4th) he showed great antipathy to running and when forced collapsed in exhaustion. Diarrhea while running marked the collapse. The diet had not been changed and his weight stood at 16.7 K. The sight of worms in his feces prompted administration of a vermifuge. A complete rest until November 17th occasioned a weight increase to 17.1 K. and the return of his spirits and desire to run. On November 17th began the final and most strenuous period. When exercise was finally discontinued on January 2, 1926, he had run another 314 miles. Quite often during the period he would run in the course of a half-day the equivalent of climbing from Manitou to the top of Pike's Peak. Again toward the close of the period he refused to run showing diarrhea and on one occasion bloody feces. On December 21st, when his weight had reached the low point, 14.6 K., an attempt was made to support him with 50 to 100 grams of dextrose daily. This was successful in holding his weight constant and even raising it a little to 14.9 K. but was unsuccessful in warding off the antipathy to running. He also developed the irritability and "crankiness" often associated with "staleness" in athletes. Entirely at variance with his normal behavior, he would now snap at attendants on the least provocation. The post-exercise period is characterized by a gradual recovery of weight accelerated by the addition of 50 grams of meat to the diet between February 20th and May 2nd. The metabolism both absolute and referred to unit area shows a decline throughout the exercise period; however, in this respect it does not differ from that of the unexercised dogs. The same parallelism holds true in the post-exercise period. The weeks of December 20th, 27th and January 3rd probably mark a very pronounced metabolic reorganization. As mentioned above, the dog at this time showed signs of staleness and collapse. Without any objective evidence one is tempted to suggest that at this point the more vital body proteins were beginning to be utilized for fuel.

In the course of the ensuing year he took on weight becoming 20 per cent heavier than his previous high level in spite of the fact that besides the temporary addition of 50 grams of meat to the diet mentioned above there was no change in the diet. The general appearance of the animal after recovery from the immediate effects of the exhaustive training period was one of unusual muscular power. An inward rotation of the front legs indicated the increased power of the pectoral groups and gave to him the broad chest and turned-out-leg appearance of the "bull dog." Without exaggerating, it may be said that his appearance was distinctly altered. In spite of this distinct increase in musculature, 21 tests in January and

TABLE 2

Basal metabolic rate

The determinations are given in weekly averages under date of the respective Sunday.

DATE OF THE WEEK	DOG 4					DOG 5					DOG 6					DOG 7				
	Number of tests	Average calories per hour	Average weight in kilos	Calories per square meter per 24 hours*	Per cent deviation from average for normal dogs†	Number of tests	Average calories per hour	Average weight in kilos	Calories per square meter per 24 hours*	Per cent deviation from average for normal dogs†	Number of tests	Average calories per hour	Average weight in kilos	Calories per square meter per 24 hours*	Per cent deviation from average for normal dogs†	Number of tests	Average calories per hour	Average weight in kilos	Calories per square meter per 24 hours*	Per cent deviation from average for normal dogs†
May 10, 1925	7	25.5	16.8	830	+7.8	7	26.6	18.9	805	+4.5	4	29.8	25.9	730	−5.2					
17	6	25.3	17.0	820	+6.5	5	26.0	18.9	785	+1.9	5	30.2	26.1	735	−4.5	0		20.3		
24	6	26.0	16.7	855	+11.0	3	27.5	19.0	830	+7.8	1	29.7	26.0	725	−5.8	0		19.7		
31	2	25.0	16.5	830	+7.8	2	28.7	18.8	870	+13.0	2	31.9	25.9	780	+1.3	0		19.3		
							Hair clipped					Hair clipped								
June 7	4	27.3	16.9	890	+15.6	2	31.5	18.8	955	+24.0	3	34.7	26.2	845	+9.7	0		19.6		
14	4	25.1	17.1	810	+5.2	4	26.6	18.6	810	+5.2	4	35.1	26.4	850	+10.4	0		19.6		
21	6	25.7	17.3	825	+7.1	4	27.8	18.6	850	+10.4	3	34.6	26.6	830	+7.8	0		19.7		
		Exercise begun																		
28	6	26.0	17.4	830	+7.8	4	27.5	18.7	835	+8.4	3	35.3	26.8	845	+9.7	0		19.6		
July 5	6	24.6	17.4	785	+1.9	4	28.2	19.0	850	+10.4	6	33.4	27.0	795	+3.2	0		19.8		
							Exercise begun					Exercise begun								
12	7	25.2	17.3	810	+5.2	4	30.0	19.2	895	+16.2	4	34.6	27.2	820	+6.5	3	29.8	20.1	865	+12.3
19	6	25.4	17.3	815	+5.8	4	31.3	19.3	930	+20.8	4	35.7	27.2	845	+9.7	4	31.6	20.1	915	+18.8
26	6	25.5	17.6	810	+5.2	3	31.7	19.3	945	+22.7	2	36.1	27.0	860	+11.7	5	33.2	20.4	950	+23.4
August 2	7	26.2	17.5	835	+8.4	5	31.2	19.2	935	+21.4	0		27.4			5	32.6	20.6	930	+20.8
9	7	25.3	17.6	800	+3.9	4	27.2	19.2	815	+5.8	2	34.4	28.0	800	+3.9	3	30.1	20.8	850	+10.4
16	6	24.8	17.6	785	+1.9	5	27.7	19.2	830	+7.8	1	30.3	28.2	700	−9.1	2	30.3	21.0	855	+11.0
23	7	26.0	17.7	820	+6.5	3	29.2	19.2	875	+13.6	3	35.8	27.9	835	+8.4	3	29.6	21.0	835	+8.4
30	7	24.6	17.9	770	0.0	3	28.0	19.0	845	+9.7	3	33.2	27.8	775	+0.6	3	28.9	21.2	810	+5.2
September 6	7	23.7	18.0	740	−3.9	6	26.5	19.1	795	+3.2	6	33.2	27.6	780	+1.3	7	28.8	21.2	805	+4.5
13	5	24.7	18.0	770	0.0	6	24.4	19.2	730	−5.2	5	30.4	27.6	715	−7.1	6	29.4	21.4	815	+5.8
							End of exercise					End of exercise								
20	6	25.1	17.8	790	+2.6	0		19.3			3	30.6	27.6	720	−6.5	0		21.4		
27	5	25.2	17.8	790	+2.6	1	24.8	19.7	730	−5.2	2	32.0	27.9	745	−3.2	2	29.6	21.4	825	+7.1
October 4	5	26.7	17.8	840	+9.1	2	29.9	19.7	880	+14.3	2	32.3	27.8	755	−1.9	1	32.4	21.2	905	+17.5
11	5	25.1	17.6	795	+3.2	2	26.2	19.7	770	0.0	1	35.6	27.7	835	+8.4	0		21.1		
18	7	23.8	17.5	755	−1.9	2	26.7	19.8	785	+1.9	2	30.4	27.5	715	−7.1	2	28.5	20.9	805	+4.5
25	7	23.0	17.2	740	−3.9	3	24.6	19.9	720	−6.4	2	32.6	27.3	770	0.0	2	29.6	20.9	835	+8.4
November 1	4	23.5	16.7	770	0.0	0		20.0			3	30.8	27.3	730	−5.2	1	25.6	21.0	720	−6.5
		Exercise discontinued																		
8	7	22.0	16.9	715	−7.1	3	27.2	20.0	790	+2.6	1	29.5	27.5	695	−9.7	2	25.1	20.9	710	−7.8
		Exercise continued																		
15	7	23.1	16.9	750	−2.6	1	24.5	20.2	710	−7.8	2	30.9	27.3	730	−5.2	3	27.3	20.8	775	+0.6
22	7	22.5	16.3	750	−2.6	1	25.9	19.8	760	−1.3	3	29.4	27.0	700	−9.1	2	27.8	20.9	785	+1.9
29	7	21.9	15.6	750	−2.6	1	23.0	19.6	675	−12.3	2	30.6	26.8	735	−4.5	2	25.5	21.0	720	−6.5
December 6	7	21.4	15.3	745	−3.2	2	23.7	19.7	695	−9.7	2	30.4	26.6	730	−5.2	3	27.3	21.1	765	−0.6
13	7	21.7	14.9	770	−0.0	2	23.5	19.9	685	−11.0	3	28.3	26.6	680	−11.7	2	28.2	21.3	785	+1.9
20	7	19.6	14.8	695	−9.7	3	23.4	20.1	680	−11.7	2	27.6	26.4	670	−13.0	1	24.9	21.4	690	−10.4
27	6	22.6	14.9	800	+3.9	4	24.1	20.2	695	−9.7	2	29.1	26.2	705	−8.4	1	27.7	21.4	770	0.0
		End of exercise																		
January 3, 1926	5	23.9	14.8	850	+10.4	5	24.4	20.4	700	−9.1	1	28.8	26.3	695	−9.7	1	26.3	21.5	730	−5.2
10	4	21.6	15.0	760	−1.3	6	23.4	20.4	670	−13.0	2	30.0	26.2	730	−5.2	2	27.4	21.5	760	−1.3
17	5	21.2	15.2	740	−3.9	5	23.2	20.4	665	−13.6	1	29.7	26.3	720	−6.5	2	27.5	21.4	765	−0.6
24	5	23.5	15.4	810	+5.2	5	24.2	20.4	695	−9.7	2	28.1	26.3	680	−11.7	1	25.8	21.3	720	−6.5
31	5	22.7	15.7	775	+0.6	7	23.6	20.5	675	−12.3	2	27.2	26.7	650	−15.6	2	26.7	21.5	740	−3.9
February 7	3	21.9	15.9	745	−3.2	3	25.1	20.5	720	−6.5	1	26.1	26.4	630	−18.2	1	28.7	21.7	790	+2.6

* Calculated from calories per hour and weight by use of the graphic calculating device (see fig. 3) based on Meeh's formula. The maximum error is ±0.75 per cent.

† Deviations are calculated from 770 as representing that multiple of five which is nearest to the average of normal dogs as determined by Kunde and Steinhaus (1926).

February of 1926 average 738 Cal. per sq. m. Eleven in March and April average 706.8. Twelve in May and June average 758, and eleven in July

TABLE 3

Basal metabolic rate

The determinations are given in weekly averages under date of the respective Sunday.

DATE OF THE WEEK	DOG 5					DOG 6					DOG 7				
	Number of tests	Average calories per hour	Average weight in kilos	Calories per square meter per 24 hours*	Per cent deviation from average for normal dogs†	Number of tests	Average calories per hour	Average weight in kilos	Calories per square meter per 24 hours*	Per cent deviation from average for normal dogs†	Number of tests	Average calories per hour	Average weight in kilos	Calories per square meter per 24 hours*	Per cent deviation from average for normal dogs†
June 20, 1926	6	24.6	22.1	670	−13.0	4	30.3	27.3	715	−7.1	4	26.4	22.1	720	−6.5
27	7	23.4	21.7	645	−16.2	7	29.4	26.7	705	−8.4	4	26.5	21.9	725	−5.8
July 4	7	24.7	21.7	680	−11.7	7	28.8	26.7	690	−10.4	3	27.4	22.0	745	−3.2
11	6	23.1	21.8	635	−17.5	6	28.5	26.9	680	−11.7	2	28.8	21.9	785	+1.9
18	7	25.1	21.8	690	−10.4	7	29.5	26.8	705	−8.4	4	27.5	22.0	750	−2.6
25	7	26.0	21.9	710	−7.8	7	31.1	27.1	740	−3.9	4	29.0	22.0	790	+2.6
August 1	7	25.7	22.2	700	−9.1	7	30.2	27.6	710	−7.8	4	27.2	22.0	745	−3.2
8	7	26.0	22.4	700	−9.1	7	29.3	27.8	685	−11.0	3	27.1	22.1	740	−3.9
15	6	25.7	22.6	690	−10.4	7	29.7	28.0	690	−10.4	3	28.8	22.1	785	+1.9
							Exercise begun					Exercise begun			
22	7	26.1	22.7	700	−9.1	7	30.7	28.2	710	−7.8	3	29.5	22.1	805	+4.5
29	7	26.0	22.7	695	−9.7	6	32.2	27.7	755	−1.9	2	26.6	22.1	725	−5.8
September 5	6	25.5	22.6	685	−11.0	7	31.0	26.9	740	−3.9	3	28.6	21.9	785	+1.8
12	5	25.7	22.2	700	−9.1	6	28.9	27.0	690	−10.4	3	27.6	21.8	760	−1.3
												End of exercise			
19	6	25.5	22.4	685	−11.0	7	28.7	27.0	685	−11.0	3	25.6	21.8	705	−8.4
26	6	25.6	22.5	690	−10.4	6	29.6	26.9	705	−8.4	3	28.5	21.8	785	+1.9
October 3	5	25.3	22.6	675	−12.3	7	29.5	27.0	705	−8.4	4	25.0	21.8	685	−11.0
10	7	25.9	22.5	695	−9.7	7	29.8	26.8	715	−7.1	1	25.3	21.6	700	−9.1
17	6	26.3	22.5	710	−7.8	6	29.6	26.8	710	−7.8	3	27.0	21.8	740	−3.9
24	7	25.9	22.5	695	−9.7	7	29.9	26.8	715	−7.1	4	27.0	21.8	740	−3.9
31	6	25.3	22.4	680	−11.7	6	29.0	26.6	695	−9.7	3	26.6	21.8	730	−5.2
November 7	7	23.7	22.4	640	−16.9	6	27.2	26.3	660	−14.3	3	25.7	21.6	710	−7.8
14	7	24.2	22.5	650	−15.6	7	28.3	26.3	685	−11.0	4	28.5	21.6	790	+2.6
21	6	22.7	22.4	610	−20.8	6	27.5	26.2	670	−13.0	3	26.1	21.4	725	−5.8
28	7	24.1	22.5	650	−15.6	6	27.6	26.0	675	−12.3	5	26.2	21.5	725	−5.8
December 5	6	24.1	22.5	650	−15.6	6	26.3	25.9	645	−16.2	4	25.9	21.6	715	−7.1
							End of exercise								
12	6	24.0	22.5	645	−16.2	7	28.3	25.8	695	−9.7	4	27.3	21.6	755	−1.9
19	2	25.0	22.6	670	−13.0	2	27.3	25.9	670	−13.0	1	26.3	21.6	725	−5.8
26	2	22.8	22.5	615	−20.1	3	27.5	26.0	670	−13.0	5	27.7	21.8	760	−1.3
January 2, 1927	0		22.5			0		25.9			0		21.7		
9	0		22.5			0		25.8			0		21.5		
16	5	23.7	22.5	635	−17.5	7	28.5	25.8	700	−9.1	7	28.1	21.6	775	+0.6
23	6	23.5	22.6	630	−18.2	7	28.3	25.8	695	−9.7	7	26.0	21.6	720	−6.5

For footnotes see table 2.

and August average 735. Unfortunately a highly proliferous, condylomatous growth developed on his penis during the year and periodically gave

him great pain thus making consistent metabolic testing impossible. He is therefore used only in the data of table 2 and on the graph in figure 1.

Dog 5 is a thick matted short-haired, tan colored, male mongrel whom we secured and caged March 24, 1925. He was immediately placed on the diet indicated in table 1. No changes have been made. Successful metabolism tests were made beginning April 5th. On June 5th his hair was clipped. On July 11th, after 15 weeks of inactivity and 68 metabolism tests, his exercise was begun and continued for ten weeks. During the period he ran a total of 199.2 miles and held an almost constant weight (19.2 K.), just a little higher than the pre-training period. In the post-exercise period he started a slow but steady increase in weight which con-

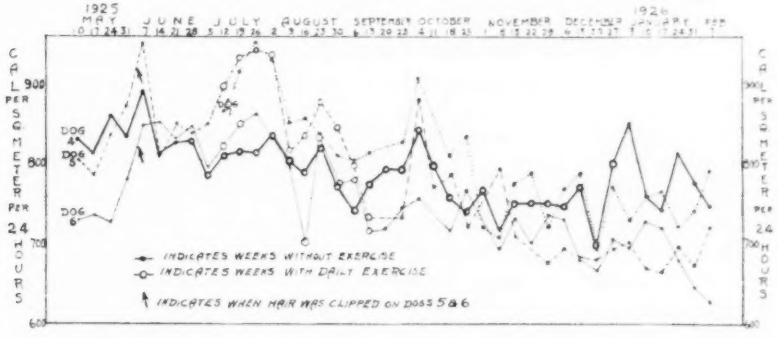

Fig. 1. The averages of all metabolism tests for each week are plotted under date of the corresponding Sunday.

Dog. 4. Inactive 14 weeks previous to July 2nd, then exercised for weeks indicated. Intensity of exercise gradually increased to 8 miles per day. Total exercise 898.0 miles. Exercise discontinued temporarily on November 4th and permanently on January 2nd because of exhaustion and "staleness."

Dog 5: Inactive 16 weeks previous to July 11th, then exercised for weeks indicated. Total exercise 199.3 miles.

Dog 6: Inactive 16 weeks previous to July 10th, then exercised for weeks indicated. Total exercise 136.9 miles.

Dog 7: Inactive 8 weeks previous to July 12th and throughout the period shown.

tinued reaching 23.6 K. in the summer of 1927. Since September 1925, this animal has been used as an inactive control. His data appear in tables 2 and 3 and in the graphs of figures 1 and 2. In spite of a full two years of inactivity the animal at the present writing shows every indication of excellent health. Though not as frisky as animals in training he is alert and quick to respond to any attention which is shown him. When not addressed he will lie at the bottom of his cage for hours at a time. His metabolism showed periodic high spots in the weeks immediately following the clipping of his hair. The summer of 1925 turned out much cooler

than anticipated when the hair of this and dog 6 was clipped. During the ten weeks of exercise he became what might be called a well-trained athlete capable of running easily three to four miles a day on a 22 per cent grade at a rate of a mile in 14 minutes. The metabolism both during and following the training period follows consistently that of the other dogs whether they are in training or out. The animal displays a striking peculiarity in body temperature. He occasionally registers marked subnormal temperature. As low as 36° has been recorded. These subnormal readings often are secured under conditions which in other dogs would show an increase in temperature; however, this is not to be associated with the long period of inactivity since it was noted as early as three weeks after the closing of his training period in 1925.

Dog 6. This is a large brown and white male of the shepherd dog type. On March 24, 1925, he was secured and caged. His weight rose quickly from 24.9 to 28.4 K. on varying diets. In August the diet was finally adjusted to the one described in table 1. On June 5th his hair was clipped and July 10th, after 15 weeks of inactivity and 46 metabolism tests, his first exercise period was begun. The training was interrupted from July 27th to August 14th, because of a leg infection. It closed September 12th with a total of 136.9 miles to his credit and his weight a little above the pre-training level. He gave every appearance of being in good training. For the succeeding 35 weeks, until May 12, 1926, he was inactive, his weight, having fluctuated over the range of about 1 kilo during the winter, was back to 27.3. Then started the second exercise period which was brought to an abrupt end on June 11th when an intestinal upset caused by mouldy bread complicated matters. A total of 98.4 miles was run in the 31 days. During this period the diet was increased to contain 147½ grams of bread and 250 grams of meat and his weight dropped about 0.5 K. This exercise period is not recorded in the tables and charts here presented, but it should be mentioned that the metabolism of the month was consistently 9 to 11 per cent below that of the average for normal dogs and very similar to that of the weeks immediately following which are given in table 3. During the week in which he suffered a mild intestinal upset from the mouldy bread, the metabolism rose to 770 Cal. which is the average for normal dogs. We feel that this observation may furnish a clue to one of the causes for the low metabolic rates secured in these experiments since our food usually was free from any contamination and that of the average dog fed on scraps is not.

After a return to the standard diet for two weeks the diet was again increased to 250 grams of meat and 95 grams bread. This brought his weight up rapidly to 28.2 K. Then on August 22nd he was returned to the standard diet and the third exercise period was begun, 10 weeks of inactivity having intervened since the last exercise period. Since in very

warm weather it was sometimes difficult for a dog to manage increased diet and exercise simultaneously, this attempt to first fatten an animal was tried out. The third exercise period may be termed as milder. A total of 106.5 miles was run in the 16 weeks which closed on December 9th. However, the tread was now running a mile in 12 minutes whereas previously the mile was run in 14 minutes. The exercise brought his weight down to 25.8 K. He was continued on the standard diet and returned to 27.0 K. at the end of March 1927. The data of the first training period appear in table 2 and figure 1. That of the third period appears in table 3 and figure 2. The metabolism throughout the three training periods and the pre- and post-training periods follows that of the other dogs. As in the case of dog 5, a number of high runs in the early part of the summer of 1925 may be attributed to the fact that his hair had been clipped.

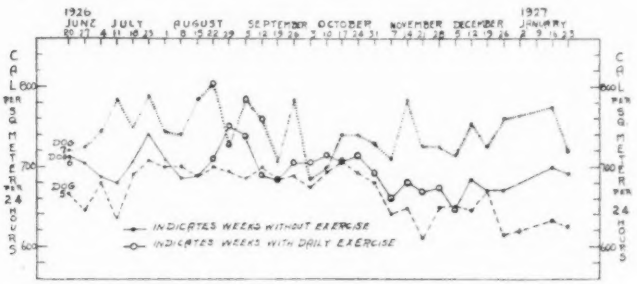

Fig. 2. The averages of all metabolism tests for each week are plotted under date of the corresponding Sunday.

Dog 5: Inactive through period shown and for 46 weeks preceding June 20th.

Dog 6: Inactive for 10 weeks preceding August 22nd, then run a total of 106 miles in the weeks indicated.

Dog 7. Inactive for 66 weeks preceding August 22nd, then run a total of 12.5 miles in the weeks indicated.

Dog 7 is a short-haired, brown and white mongrel of the bull terrier type. He was secured and caged May 21, 1925. He was immediately placed on the standard diet indicated in table 1, and this continued unchanged throughout the period covered by these experiments. On August 22, 1926, after 66 weeks of inactivity, he was run ½ mile per day or a total of 12½ miles in 27 days. His weight varied very little throughout the period here recorded. Though inactive for a long time he never showed signs of flabbiness, being always easily excited. He has shown considerable stubborness when attempts were made to exercise him. Only recently has this been overcome in using him in connection with another phase of our work. He may well be considered as an inactive control for the periods covered by both tables 2 and 3.

DISCUSSION OF RESULTS. *Increased musculature.* The data present no direct evidence that exercise increases basal metabolism by increasing the bulk of the musculature. If the gain in weight in dog 4 which came on during the training period (table 2) and persisted through 16 weeks of running may be assumed to represent increase in musculature, then it would appear that such is not the case. One must not forget that a most characteristic sign of the trained athlete is his control of his muscles. *This means the ability to relax as much as it does the ability to contract.* The complete relaxation of muscle groups not at the moment engaged is the perfection of reciprocal innervation, is the athlete's most important accomplishment in the delay of fatigue, and distinguishes him more readily from the untrained than does his ability to contract muscles. It is common knowledge that one relaxes readily after vigorous physical work. Without exception in this work, the dogs in training relaxed more readily when being tested than those who were not in training.

Without committing oneself to Cathcart's notion (1922) that muscle tonus is the product of protein metabolism in the muscle sarcoplasm, one can agree with him that the maintenance of muscle tonus represents the chief O_2 requirement under basal conditions. It does not seem unreasonable to assume that in a period of training muscle bulk may increase but there may be a simultaneously established economy represented by decreased muscle tone during rest. This view is consistent with the findings of Schneider et al. (1927) already mentioned, and does not deny the possibility of a greater metabolism in a very muscular individual, compared with a "non-muscular" one when both have their muscles in comparable physiologic states. We must, in evaluating such data as are presented by Benedict and Smith (1915) also face the possibility that higher metabolism due to any of a number of possible factors may be a cause for, rather than a result of, athletic prowess.

It is true that on the diet restrictions observed in these experiments there may not have been ideal conditions for muscle growth in all cases. Another set of experiments in which dietary increases are made are under way and may throw some light in this direction.

After-effects. Any change in metabolism due to after-effects of exercise would, by definition, intimate a failure to approximate basal conditions within the time allowed to elapse since the last exercise. In the training periods as they were staged in these experiments, after-effects should display themselves by rises in the metabolism which would wear off as the animal became better trained or if they did not wear off in the course of the exercise period then by a sudden drop at the end of the period. With the possible exception of dog 6 in figure 2, we have no curve which answers this description and even here the rise in metabolism at the beginning of the training period is not without parallel in the pre-training period of

the same dog and in the controls. "After effects" of exercise may possibly be due to: 1st, oxygen necessary for oxidative removal of lactic acid; 2nd, the specific stimulating power of lactic acid as a hydroxy-acid; 3rd, the reflex stimulation of other muscle groups from soreness in muscles or joints; 4th, specific dynamic effects of proteins involved in tissue catabolism when exercise is carried to this point. It is of interest that the most clear cut illustration of "after-effects," namely, the work of Wishart (1927), is based on work done on a hand ergometer. His subjects performed 25,000 kgm. of work in an hour. It is just this type of work that would be expected to produce soreness in muscles and joints whereas the types of exercise used in the work of Hill (running) and Schneider (athletic training) by distributing the work generally to all muscles of the body would produce soreness only when driven to extreme or when required of the very flabby without systematic training. Mention should here be made of Kunde and Nordlund's dog II (1927). As described in their article and as confirmed by personal communication with Doctor Kunde, this dog was utterly unfit for exercise. For weeks he had been carried between his cage and the metabolism room. Doctor Kunde has described him as very obese and agrees that if the dogs here described are inactive and comparable to men in sedentary occupations, then her dog would classify as a patient, bedridden with obesity. It is not at all improbable that such an animal would show increased metabolism due to "after-effects" as here defined. However, this cannot be substantiated by the method suggested above for recognizing "after-effect" because there is no after-training period. The dog died while yet in the exercise period. Furthermore, our data on dog 6 (table 3) show that it is possible to thoroughly train a dog who at the start of training has a metabolism of −10 per cent so that he will have a still lower metabolism (−16 per cent) at the end and, as will be made clear later, without holding the exercise responsible for the drop. A similar drop from +10 per cent to −5 per cent was observed on dog 5 (table 2).

Variations in and out of training. The data here presented lend *no* support to the view that a period of exercise increases the metabolic level during the training period and for some time after. The work of Lusk (1915) and Lusk and DuBois (1924) is most outspoken in this respect. We have been able to duplicate their results on dogs *in the absence of exercise.* Thus dog 5, in the week of May 23, 1926, (data not given elsewhere in this paper) averaged in seven tests 635 Cal. per unit surface per day. The same average resulted from six tests in the week of July 11th (see fig. 2). From then on the metabolism remained close to 700 reaching 710 in the week of October 17th and then declining to establish a lower level in November and December. But the dog was not exercised nor was he sent to the country. He remained in the same cage he had occupied all year and served as an inactive control. A somewhat similar

curve, though not as pronounced, is found in the records for dogs 6 and 7 for the same summer.

Normal variations. We believe that none of our data points to positive effects of exercise on basal metabolism. As was pointed out above, the literature is rich with observations on the wide range over which normal basal metabalism may vary. Our data furnish no exceptions. We have been successful by the measures adopted to secure basal metabolisms in normal, well nourished, active, and inactive dogs, consistently lower than those reported by any other workers with normal material. We believe it is only from such a low level base line that the influence of various factors may be determined and then only when these factors are one at a time sufficiently isolated from all others and when safeguards are set up to rule out with suitable "controls" the influence of all other uncontrollable factors. It is also to be expected that such dogs will show wider variations in their metabolism in response to various factors than will animals who are constantly living at a higher level in which many factors are already exerting their actions and reactions, the algebraic sum of which constitutes the metabolic rate. It was at the close of Professor Cathcart's (1922) address before the British Medical Association at Glasgow in July of 1922 that Dr. J. B. Orr of Aberdeen is reported by the Secretary of that body to have commented that, "The theoretical lowest level of metabolism was unattainable under experimental conditions. . . . . True basal metabolism was an abstraction. What we really studied was different levels of metabolism with varying degrees of intensity of many functional activities. In studying the influence of any factor in the rate of metabolism, it was of course necessary to have a fixed base line, but that was merely an arbitrary condition where, as far as possible, the different factors that influenced metabolism were controlled preferably at a low level and specified."

We believe that at present the conclusion is warranted that our animals were subject to variations in basal metabolism due to factors as yet un-

Fig. 3. A calculating device for graphically determining surface area and calories per square meter per twenty-four hours from weight and calories per hour in the dog.

To use the calculating device. From the point representing weight in kilograms on the vertical scale project horizontally to the intersection with the curved line indicated by $0.112\sqrt[3]{W^2}$. This intersection projected to the horizontal scale at top or bottom reads the surface area in meters to the third decimal point. To determine calories per square meter in 24 hours return to the intersection of the weight line with the curved line and now project vertically upwards or downwards to the horizontal line representing calories per hour read from the *same* vertical scale as that from which the weight was read. Mark this second intersection and referring to the scale represented by the diagonal lines read the calories per square meter per twenty-four hours. This last scale is graduated in twenty calory intervals. Within this interval it is safe, in most positions on the graph, to estimate to the nearest multiple of five. In actual practice the operation of the device incurs no error greater than ± 0.75 per cent.

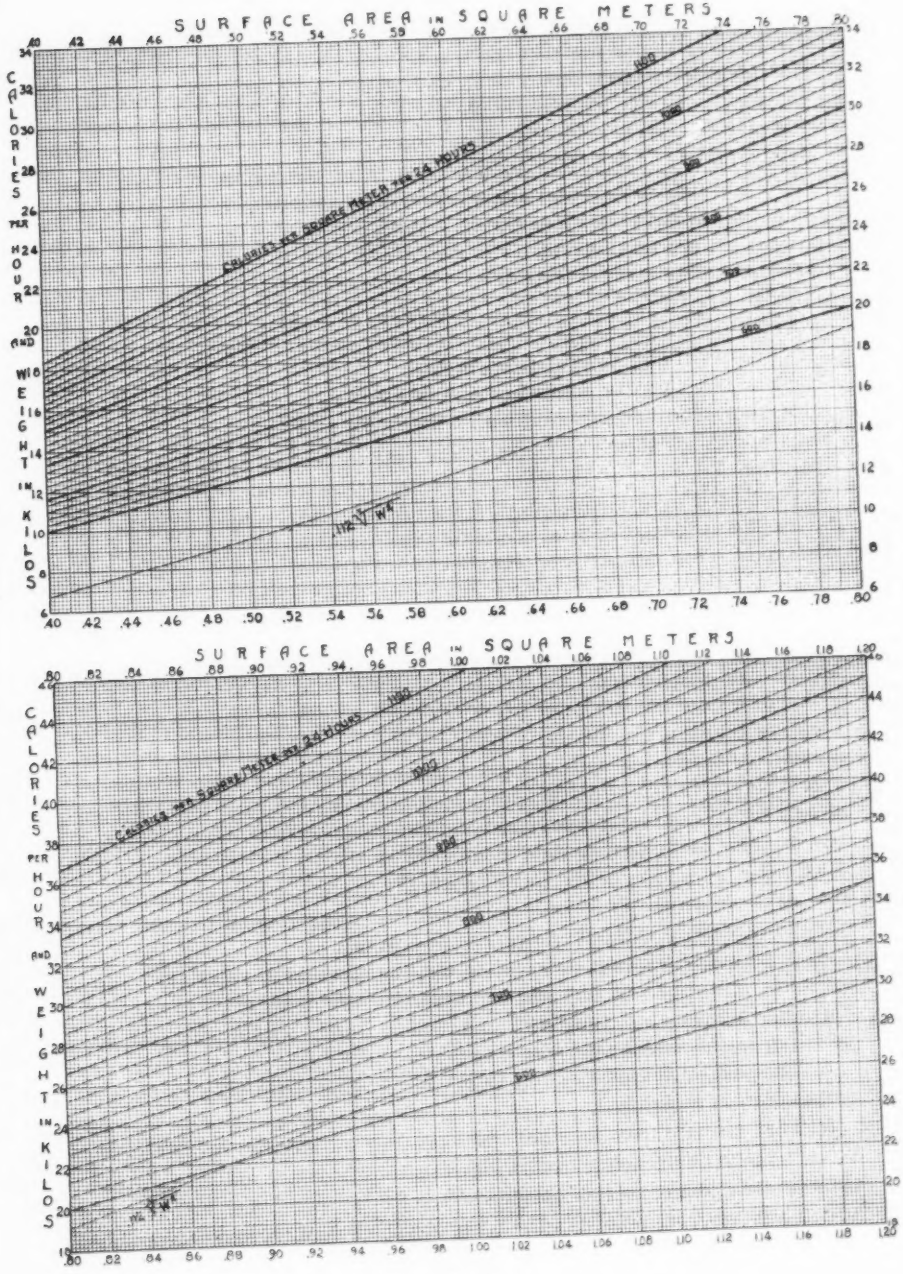
SURFACE AREA IN SQUARE METERS
CALORIES PER HOUR AND WEIGHT IN KILOS
CALORIES PER SQUARE METER PER 24 HOURS
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
$W^{\frac{2}{3}}$
$W^{\frac{1}{3}}$
$W^{\frac{1}{4}}$

Fig. 3

known to us. That these variations are undisturbed by activity and inactivity, and that if activity and inactivity as here defined and developed does affect the metabolism, it is in such minute amount or in so gradual a fashion as not to be made out with any degree of positiveness in the period here under observation. We are not unmindful of the fact that our dog seven who has had the least exercise is showing the highest metabolic rate referred to unit surface and also that he has failed to gain weight as pronouncedly as have the other three. Continued observations will clear this point. We are also hopeful of being able to show with further observations that there is some order in what at present must be called "spontaneous" variations in basal metabolism. Lindhard (1912) and Griffith (1926) and others have suggested seasonal correlations and Lindhard believes that the quantity of light is a causative factor.

We believe that the method of comparing the average of a series of consecutive tests from one period with a like series from another period in the same animal as seems to be common practice is inadequate and is responsible for some of the contradictions in the literature. It must be supplemented by comparisons with data from normal control animals run under identical experimental conditions and *simultaneously* to rule out the so-called "spontaneous" variation.

SUMMARY

1. The possible ways in which exercise may modify the basal metabolism are outlined and defined.

2. For an answer to the question of whether exercise may increase the basal metabolism via the route of increasing the musculature in an adult animal, our data are insufficient but indications point to a negative answer.

3. The possibility of an increased resting metabolism due to a residual after effect of exercise is recognized and not unexpected in cases of strenuous exercise of limited muscle groups or of the extremely flabby. By taking the precaution of starting in gradually as observed in this investigation, it is shown to be possible to train a sedentary animal to take very strenuous exercise without showing after-effects.

4. A period of training sufficient to put the musculature and the entire organism into excellent physical condition for strenuous work is without apparent influence on the basal metabolism either within the training period or in the subsequent post-training period.

5. The possibility that as a long term effect strenuous training may lower metabolism is recognized.

6. That exercise may in other ways produce an increase in the nutritional economy evidencing itself in increased weight is also indicated by the data as deserving of further consideration.

7. There are fluctuations in the basal metabolism of normal dogs which

at present had best be termed "spontaneous". Seasonal factors may play a part.

During the period from March to September, 1925, R. E. Johannesen assisted in the technical work of this experiment. In the period from October 1925 to June 1926, G. G. Crisler assisted. From July 1926 to January 1927, T. A. Jenkins did the same.

BIBLIOGRAPHY

BENEDICT, F. G. 1915. Journ. Biol. Chem., xx, 263.
BENEDICT, F. G. AND T. M. CARPENTER. 1910. Carnegie Inst. Wash. Pub. 126, 236.
BENEDICT, F. G. AND E. P. CATHCART. 1913. Carnegie Inst. Wash. Pub. 187, 171.
BENEDICT, F. G. AND H. M. SMITH. 1915. Journ. Biol. Chem., xx, 243.
BOOTHBY, W. M. AND I. SANDIFORD. 1924. Physiol. Rev., iv, 81.
BUGBEE, E. P. AND A. SIMOND. 1926. This Journal, lxxxv, 542.
CATHCART, E. P. 1922. Brit. Med. Journ., ii, 747.
COWGILL, G. R. AND D. L. DRABKIN. 1927. This Journal, lxxxi, 36.
GRIFFITH, F. R., G. W. PUCHER, J. D. KLEIN AND M. E. CARMER. 1926. Proc. Soc. Exper. Biol. and Med., xxiii, 464.
HARRIS, J. A. AND F. G. BENEDICT. 1919. Carnegie Inst. Wash. Pub. 279, 244.
HILL, A. V. 1926. Muscular activity. The Williams & Wilkins Co., 94.
KUNDE, M. M. 1923. Journ. Met. Res., iii, 399.
KUNDE, M. M. AND A. H STEINHAUS. 1926. This Journal, lxxviii, 127.
KUNDE, M. M. AND M. NORDLUND. 1927. This Journal, lxxx, 681.
LINDHARD, J. 1912. Skand. Arch. f. Physiol., xxvi, 221.
LUSK, G. 1915. Journ. Biol. Chem., xx, 555.
LUSK, G. AND E. F. DUBOIS. 1924. Journ. Physiol., xlix, 213.
PALMER, MEANS AND GAMBLE. 1914. Journ. Biol. Chem., xix, 239.
ROTH, P. 1922. Boston Med. Surg. Journ., clxxxvi, 491.
SCHNEIDER, E. C., R. W. CLARK AND G. C. RING. 1927. This Journal, lxxi, 255.
STEINHAUS, A. H 1926. This Journal, lxxvi, 184.
WISHART, G. M. 1927. Quart. Journ. Med., xx, 193, 199.
ZUNTZ, N. 1903. Pflüger's Arch., xcv, 192.
ZUNTZ AND SCHUMBERG. 1901. Physiologie des Marches. Berlin, 216.

THE EFFECT OF EXERCISE UPON DIASTOLIC HEART SIZE

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Received for publication October 21, 1927

For a considerable number of years the question of the influence of exercise upon the size of the heart has been a debated one. A large clinical literature prior to the introduction of the x-ray as a means of study of the heart supported the idea that the heart was dilated by exercise.

With the exception of Schott and Savage (1912), who found that after exercise there was a dilatation, and Boigey (1921), who found that with severe exercise continued more than 15 minutes a dilatation resulted, all other workers using the x-ray method of study have reported either no change or a decrease in heart size following exercise of various types and severity. These latter investigators include Hoffmann (1902), de la Camp (1903), Lennhoff and Levy-Dorn (1905), Levi (1906–07), Kienbock, Selig and Beck (1907), Mendel and Selig (1907–08), Dietlen and Moritz(1908), Raab (1909), Williamson (1915) and Bordet (1923). It is of interest to note that although Bordet found no change in cardiac area after exercise, he believes that the hearts of subjects examined orthodiagraphically had an increased output, that is to say, greater diastolic inflow as shown by the amplification of the beats. He believes that the stroke volume is augmented during systole, which results, even with a decreased cardiac area, in an increased cardiac output.

Attempts to ascertain whether there is any change in the size of the heart due to exercise are of course incomplete unless they are made while the exercise is in progress. After the cessation of exercise the heart will return to its original size very quickly. According to Nicolai and Zuntz (1914), this may occur in 3 to 4 seconds after discontinuing exercise.

Moritz (1908) obtained x-rays of 11 subjects during static exercise. The heart was found to be decreased in size during this type of effort. He states that even pathologically dilated hearts may undergo diminution of area.

Obviously this type of exercise may give entirely different results from those obtained during dynamic exercise since, in the former, muscular contractions which aid venous return are entirely absent, save at the instant of the initial effort, while in the latter the pumping action of the contracting muscles is an active accessory to the circulation.

More to the point in our investigation are those observations carried out during dynamic exercise. Nicolai and Zuntz (1914) studied four subjects who worked on a treadmill. The cassette was strapped to the chest, and an upright standard was placed in front of the subject so that at the instant the exposure was made the subject could walk up against this standard, and thus be at the same distance from the tube each time the teleroentgenogram was taken. Exposures were made without interrupting the exercise. The work done by the subjects was moderate in extent, since the rate of walking was approximately three miles per hour, and the incline of the treadmill was 14°. The teleroentgenograms were taken at a distance of 1 meter from tube to plate, without correction for distortion. The transverse diameter of the silhouette alone was measured and cardiac volume computed from these data. They found a slight increase in transverse diameter during exercise (3 mm.) and a somewhat greater decrease in transverse diameter a few seconds after the cessation of exercise.

Bruns (1921) reported 46 subjects whose exercise consisted of alternately flexing and extending one leg while in the erect position. The heart was studied orthodiagraphically at intervals as frequent as one minute, the exercise being continued until the subject was fatigued. During the experiment each subject was supported at the small of the back to prevent too great body movement at the time the orthodiagrams were being made. There was never any appreciable increase in the cardiac area during exercise even when the effort was severe enough to increase the pulse rate as much as 10 to 50 beats per minute and blood pressure 10 to 40 mm. of Hg. He was able to show in some subjects a diminution in the size of the heart during exercise.

In experiments which were designed primarily to study cardiac output in rest and in exercise Meek and Eyster (1922) report 17 subjects where teleroentgenograms were made in both systole and diastole during rest and mild exercise. The exercise consisted of lifting with each hand a 2 kilogram weight from a hanging position at the side of the body to the height of the shoulder at a moderate rate of speed, while the subject sat in a chair. When the teleroentgenograms were taken the subject leaned forward slightly against a heavy base stand which carried the films. The tube was placed a distance of 1 meter from the film, and correction was made for distortion of the silhouette. They found that in 7 of the 17 subjects there was a diminution of diastolic size, while in 10 there was an increase, the changes being slight in all cases.

Method. A bicycle ergometer with a prony brake by which tension on the rear wheel could be maintained at any desired point was used. The machine is essentially that described by Sevringhaus (1927), except that we have used a spring balance instead of the Harvard balance to determine load.

The subjects are divided into three groups, on a basis of the present physical training of the subjects. The first comprises 8 male staff members and medical students between the ages of 23 and 31 years, who were not in training.

The second consists of two individuals, P. M. D., trained in cycling of this type by the continued use of such an apparatus for his own exercise, and one untrained subject, C. W. M. Both rode with a 2.7 kgm. load, P. M. D. riding 30 minutes, C. W. M. riding 25 minutes.

The third group comprises 8 specially selected girls, aged 19 to 30 years, from the classes of Physical Education, who were active in at least two branches of athletics, and who were therefore in training, although not necessarily cyclists.

The subject sat erect on the machine, the erect position being maintained during the time of riding by means of two adjustable rods with padded U shaped ends, supported upon an adjustable frame which was bolted to the base of the apparatus. The U shaped end of the rods were fitted into the axillary border of the shoulder in such a way as to bring the cassette into a vertical position relative to the floor. Careful adjustment of the rods was made to prevent compression of the axillary vein at the point of application

The cassette was strapped by means of an adjustable harness over the anterior thoracic wall so that it would move from side to side with the thorax in such movements as were made in exercise. Teleroentgenograms were made with the tube 2 meters distant from the calculated anterior margin of the heart as advised by Bardeen (1918). The subject sat erect, since it has been shown by Hodges (1924) that in 11 per cent of normal subjects the heart shadow is enlarged when the body is inclined forward. The exposures were made first with the subject at rest, seated on the bicycle, and at such time as the venous pressure and pulse rate had become constant, then during exercise, and approximately 20 seconds and 5 minutes after the cessation of exercise.

Venous pressures were determined with the manometer described by Hooker and Eyster (1914), and pulse rate by palpation of the radial artery, at approximately one minute intervals before, during and after exercise, the latter being continued for from 8 to 10 minutes after the cessation of exercise. A vein on the dorsum of the left hand was used to determine venous pressure, the hand being accurately adjusted by means of a carpenter's level to the level of the costal angle in each subject. The forearm and hand rested upon an adjustable table. The venous pressures are obviously higher when so taken than the pressure at the mouth of the great veins, but the pressure is representative of that at a constant level.

The rate of pedaling was timed by a metronome to give a uniform rate of 60 revolutions of each pedal per minute, it having been shown by Bene-

dict and Cathcart (1913) that the greatest efficiency is reached at rates of from 60 to 70 revolutions of each pedal.

The cardiac silhouette areas were determined by first outlining the borders and continuing them to enclose the shadow where it was obscured by the diaphragm and great vessels. Plane area and greatest transverse diameter were measured, and all mensurations were corrected for distortion of the heart silhouette. Volume changes of the heart were computed according to changes in area, using the tables of Bardeen (1918).

In the preliminary work difficulty was encountered in the proper mensuration, especially of area, of the cardiac silhouette. The hearts most difficult to outline and measure with precision are those which lie more or less in the transverse position, and where the diaphragm is relatively high. Under these circumstances, accurate outlining of the lower border is difficult or may be impossible. The subjects included in the present work were selected to avoid this difficulty. No subject was included whose heart silhouette varied more than 2 per cent in area and more than 2 per cent in transverse diameter when outlined and measured three successive times.

In order to select proper subjects for the work, most of them were examined under the fluoroscope, and only those whose hearts had the apex and a relatively large part of the lower border free from the diaphragm were used.

We have estimated the minute volume of the heart by multiplying a systolic discharge of 1.23 cc. per kilo of body weight, by the pulse rate per minute. The figure chosen (1.23 cc.) is an average taken from the work of Howell and Donaldson (1884), Henderson and Haggard (1922 and 1925), Meek and Eyster (1923), and Collett and Liljestrand (1924). Assuming that the degree of systolic emptying, upon which however we have no data, did not change during the experiment, we estimated the changes in minute volume output on a basis of the changes in pulse rate and diastolic volume. Such a computation is by no means exact, but it is believed to indicate the direction in which events are proceeding.

Results. In table 1 appear the results of three experiments which are a fair average of the changes in heart size encountered in work of different severity.

The first subject H. H. S. exercising moderately, load 1.58 kgm., did 4905 kgm. m. of work in 10 minutes. The pulse rate was rather high, reaching a maximum of 160 per minute after he had ridden for $9\frac{3}{4}$ minutes. The maximum venous pressure, 12 cm. of water, was attained after 4 minutes of exercise. During exercise cardiac area increased 3.3 per cent, while transverse diameter remained unchanged. After the cessation of exercise, the area of the cardiac silhouette decreased 8.2 per cent, and transverse diameter diminished 7.6 per cent below the resting level. The estimated

TABLE 1

Effect of exercise upon the heart

X-RAY TIME	AGE	HEIGHT	WEIGHT	PULSE RATE	VENOUS PRESSURE H_2O	SILHOUETTE AREA	PER CENT VARIATION AREA ± NORMAL	TRANSVERSE DIAMETER	PER CENT VARIATION TRANSVERSE DIAMETER ± NORMAL	VOLUME	ESTIMATED MINUTE VOLUME	WORK
H. H. S. 10 minute moderate exercise												
		inches	*kgm.*	*per minute*	*cm.*	*sq. cm.*		*mm.*		*cc.*	*liters*	*kgm. M.*
Normal	24	69	63.5	70	4	122		132		714	4.83	4,905
8 minute exercise				150	11	126	+3.3	133		750	17.12	
20 seconds after exercise				160	11	113	−7.4	123	−7.0	637	1.76?	
5 minutes after exercise				92	7	112	−8.2	122	−7.6	628	?	
H. H. S. 10 minute severe exercise												
Normal	24	69	63.5	70	4	121		134		705	4.83	8,315
8 minute exercise				196	9	126	+5.0	133		750	24.13	
20 seconds after exercise				198	10	119		128	−4.5	688	12.10	
5 minutes after exercise				112	8	117	−3.3	127	−5.2	671	4.94	
C. W. M. 25 minute severe exercise												
Normal	31	70	65.5	96	8	138		143		860	7.74	
2 minute exercise				132	15	146	+6.2	147	+2.8	934	20.41	
7 minute exercise				176	12	145	+5.5	143		925	25.62	
16 minute exercise				200	20	145	+5.5	138	−3.4	925	29.12	
23 minute exercise				192	17	141	+2.1	138	−3.4	887	20.84	
20 seconds after exercise				192	17	135	−2.1	132	−7.5	832	10.10	
5 minutes after exercise				124	9	131	−4.8	132	−7.5	795	1.93?	
										Total work		21,250

minute volume increased 253 per cent during exercise. It will be noted that 5 minutes after exercise the estimated minute volume had diminished by more than the resting output.

The same subject doing severe exercise for 10 minutes, with a load of 2.7 kgm., showed an increase in diastolic area of 5 per cent during exercise, with no appreciable change in transverse diameter. The pulse rate was high, the maximum obtained just before the end of exercise being 198 per minute. The maximum venous pressure of 10 cm. of water was reached after 9 minutes of exercise. Five minutes after the cessation of exercise cardiac area had diminished 3.3 per cent and transverse diameter had decreased 5.2 per cent below the normal. The estimated minute volume increased 400 per cent during exercise, and it had returned to the resting level 5 minutes after exercise was discontinued. The work done amounted to 8315 kgm. m.

The second subject, C. W. M., rode for 25 minutes with a load of 2.7 kgm., at a rate of sixty revolutions of each pedal per minute. The teleroentgenograms were taken at intervals which were determined by the increases of venous pressure and pulse rate. The first taken two minutes after the ride began showed the maximum increase in the area of the silhouette which was 6.2 per cent. At this time transverse diameter had increased 2.8 per cent, the pulse rate was 132 per minute and venous pressure had increased to 15 cm. of water, increases of 36 beats per minute and 7 cm. of water. At the end of 7 minutes of exercise the area of the silhouette was 5.5 per cent greater than normal, there was no change in transverse diameter, pulse rate had further increased to 176 per minute, while venous pressure had fallen to 12 cm. of water. After riding 16 minutes, the area of the silhouette was still 5.5 per cent larger than normal, transverse diameter had decreased 3.4 per cent below normal, pulse rate was 200 per minute, and venous pressure was 20 cm. of water. The marked rise of pulse rate and venous pressure was due to the prony brake which stuck for half a minute and friction load for that time increased to 3.1 kilograms. This was then corrected to 2.7 kgm. for the remainder of the ride. At the end of 23 minutes' riding the area of the silhouette was 2.1 greater than normal, transverse diameter 3.4 per cent less than normal, pulse rate was still high, 192 per minute, and venous pressure was also high, 17 cm. of water. Twenty seconds after the subject stopped riding the cardiac area was 2.1 per cent smaller than normal, and transverse diameter was decreased below normal 7.5 per cent. Five minutes after the cessation of exercise the area of the silhouette and transverse diameter were diminished 4.8 per cent and 7.5 per cent respectively, below normal. The work done was 20,990 kgm. m. During exercise the minute volume increased until the heart reached its greatest output, after 16 minutes of exercise. The exceedingly low figure of 1.93 liters was reached 5 minutes

after the cessation of exercise. Neither of these subjects was in physical training, the only exercise taken being an average amount of walking each day.

In figure 1 are the results from the subject, P. M. D., who rides the stationary bicycle for exercise and is definitely in training for this type of work. He was allowed to set his own pace, which was found to be somewhat faster than that used by the other subjects. The load used was 2.7 kgm., the same as in the severe exercise of shorter period. He rode for 30 minutes, and teleroentgenograms were taken at the end of 14, 17, 24 and 28 minutes of exercise. At 14 minutes there was a 12.6 per cent

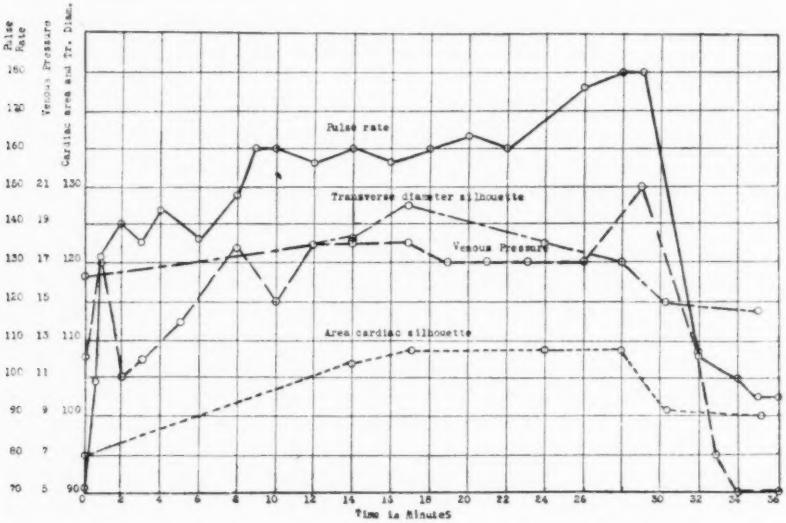

Fig. 1. Severe exercise for 30 minutes by subject P. M. D., age 53. Rate of pedaling = 148 R.P.M. Work done = 31545 kgm. m.

increase in silhouette area with an increase of 3.4 per cent in transverse diameter. Pulse rate was moderately high, 160, venous pressure high, 18 cm. At 17 minutes area had increased 14.7 per cent, transverse diamter 6.8 per cent, with pulse rate and venous pressure the same as at 14 minutes. At 24 minutes cardiac area remained 14.7 per cent above the resting area, transverse diameter was 3.4 per cent higher than normal. Pulse rate and venous pressure showed practically no change from the two preceding determinations. Immediately after the 30 minute period of exercise the area of the silhouette was increased 6.3 per cent above normal, and transverse diameter had practically returned to the normal level. Five minutes after exercise the area of the silhouette had not returned to

the resting level, but there was a 4.2 per cent diminution in the transverse diameter of the heart. The work done was 31,545 kilogram meters. The estimated minute volume showed the greatest increase after exercise had been carried on for 28 minutes. At this time it was 539 per cent higher than the resting level, to which level it had not returned 5 minutes after the cessation of exercise. In this subject the exercise was carried to the limit of effort. The two experiments reported from subjects C. W. M. and P. M. D. are the only ones in which exercise was continued more than the customary ten minute period.

It seems from the results obtained that the effect of moderate exercise on the heart during exercise is variable, and in most cases is characterized by little or no change in diastolic size. On the average, group 1, untrained subjects showed an increase of 2.5 per cent in area. Three subjects showed increases in heart size, the maximum variation being 7.5 per cent, while in three there was no change, and in two there was a diminution in cardiac area, the maximum being 5.2 per cent. After exercise was over the heart was the same size or slightly smaller. The group after exercise was over averaged a decrease in cardiac area of 3.6 per cent. The work in the 8 subjects of this group averaged 5,078 kgm. m. in 10 minutes. The estimated minute volume showed an average increase of 218 per cent during exercise.

In 6 cases of severe exercise with an average of 7,260 kgm. m. of work done in 10 minutes, all showed a slight increase in area of the cardiac silhouette. The average increase in area for the group was 4.6 per cent. Here again however the volume change was not great. The estimated minute volume increased on the average 291 per cent. One subject failed to show an increase in area during exercise, but on the contrary the heart area diminished 2 per cent. In all cases save one a slight decrease in area was noted after exercise was over, averaging 5.9 per cent for the group. The severe exercise resulted in an approach, of all subjects, to the point of physical exhaustion.

The women who were in good physical training did not differ markedly during or after exercise from the men who were not in training. Of the 8 subjects used, 4 showed an increase of 2.7 to 9.5 per cent, 3 showed no evident change, and in one there was a 5.9 per cent diminution in the area of the cardiac silhouette. The average of the whole group shows a 3.2 per cent increase in area. In 3 subjects there was an increase varying from 7.6 to 10.3 per cent, in 3 there was no change, and in 1 there was a decrease of 2.4 per cent in transverse diameter, during exercise, an average increase of 3.4 per cent in transverse diameter. The work done averaged 4,170 kgm. m. After exercise the area of the silhouette showed a decrease in all these subjects ranging from 2.5 to 9 per cent. The average diminution of area was 5.1 per cent. The transverse diameter was 3.5 per cent greater

in 1 subject, unchanged in 2 subjects, and in 4 it was decreased, ranging from 3.6 to 7.9 per cent. The average decrease in transverse diameter for the group was 2.6 per cent.

In 6 experiments on 6 trained women during severe exercise 3 showed an increase of cardiac area varying from 4.7 to 9.3 per cent, and 3 showed no change, the group averaging a 3.4 per cent increase in heart size. One subject showed an increase of 2.5 per cent in transverse diameter, the other 5 showed no appreciable change. Following the cessation of exercise all these subjects showed a diminution of cardiac area, ranging from 4.5 to 10 per cent, the average being a decrease of 6.7 per cent. Transverse diameter decreased in all subjects from 4 to 10 per cent, the average diminution being 5.6 per cent. The work done averaged 6,480 kgm. m. Due to some of the girls being light in weight a variable load was used. In all cases the subjects were almost exhausted at the end of the exercise period.

One notes then that based on percentage changes there is a slight tendency for the cardiac area and transverse diameter to increase during exercise, the greater proportion change occurring in cardiac area.

After exercise the cardiac area tends to show a greater proportional diminution than does transverse diameter. In any case these changes in either transverse diameter or area of the silhouette are slight, and the volume changes of the heart are not great.

DISCUSSION. There was a general tendency in all subjects for the venous pressure to rise rapidly at the beginning of exercise, the heart accelerating somewhat more slowly, and in the majority of subjects a relative balance between venous pressure and pulse rate was maintained during the period of exercise. As the heart accelerated there was a tendency for venous pressure to fall, while a continuous rise of venous pressure tended to result in further cardiac acceleration.

Usually also there was a tendency for the venous pressure to fall slightly during the middle period of exercise, as the heart accelerated, with the establishment of a more or less steady state of pulse rate and venous pressure for a short time, which was then followed by a second rise of venous pressure and later heart rate near the end of exercise (cf. White, 1924). This was noted whether the work done was moderate or severe.

If the pulse rate and venous pressure changes tabulated here are computed on a percentage basis, some are certain to be quite misleading. It is well known that the venous pressure, and to a lesser extent, pulse rate, are quite variable in the sitting position. If a subject having what we call a relatively high venous pressure, say 12 cm. of water, lies down and remains quiet, it will usually fall to 3 or 4 cm. Likewise all normal subjects when lying down will exhibit about the same venous pressure. The unknown factors which combine to effect venous pressure in the sitting

position evidently are subject to large individual variations, so that percentage change has not been used in following the variations of venous pressure.

When the results of moderate and severe exercise in the men are compared it is interesting to note that in every case except one, regardless of whether the heart size increased, decreased, or remained unchanged during moderate exercise, the area always increased in severe exercise, and the transverse diameter increased in 4 of the 7 subjects during exercise. In most cases the venous pressure was increased to a greater extent than in moderate exercise, and it tended to be relatively high in severe exercise, whereas the tendency was toward a relatively low, or moderately high venous pressure in moderate exercise. The pulse rate was usually high in severe exercise, and only moderately increased in moderate exercise.

In the case of C. W. M. the cardiac area decreased during 10 minutes of severe exercise, but increased in moderate effort, the pulse rate was high and venous pressure relatively low in severe exercise. In moderate exercise the pulse rate was low and the venous pressure reached the same figure as in severe exercise, while in severe exercise the pulse rate and venous pressure attained the highest levels recorded on this subject, both remaining high throughout the duration of the ride. The venous pressure which may distend the heart under one circumstance and not another is probably dependent upon other factors than the balance between pulse rate and venous pressure. One important factor in determining how high the venous pressure will rise must be pulse rate. If a certain pulse rate is capable of emptying the venous cistern at a given rate, an increase in pulse rate, other factors remaining unchanged, should lead to a more rapid emptying of this cistern, and venous pressure should decrease and the heart become smaller. In comparing a heart which has increased its rate to a high level with the same heart at a considerably slower rate, the venous pressure in both instances increasing to the same level, the rate of filling will be no more rapid, other factors remaining constant, at the high rate, than at a lower one. The time of filling, diastole of the heart, has been shortened by increasing the rate, and in consequence, one might suspect that the heart could not fill to the same extent at a high rate, as it did at a lower rate, so that the heart would necessarily become smaller. However it has been shown by Meek (1924) that in the dog's heart the extent of filling at a given venous pressure is independent of heart rate, the heart filling to the same extent under a given venous pressure regardless of whether the rate is high or low. Therefore other factors must be operative upon which at the present time there is no evidence at hand, that would result in an increase of diastolic size in one instance and a decrease in another.

CONCLUSIONS

Such variation as the heart undergoes during moderate exercise is slight. It may remain unchanged or increase or decrease slightly.

During a short period of severe exercise there is a general tendency for the cardiac area to increase, although exceptions are noted.

After moderate exercise or a short period of severe exercise the tendency is for the cardiac area and transverse diameter to decrease. The diminution is more marked after severe exercise.

During a long period of severe exercise there is greater dilatation of the heart, as compared with that in moderate exercise. The increases in transverse diameter were slight.

After a long period of severe exercise the heart may not return to its original volume 5 minutes after the exercise is over.

Minute volume increases markedly during all types of exercise used, and in some cases the minute volume may apparently diminish before exercise is finished.

After exercise is over, minute volume usually shows a very marked and rather abrupt diminution, reaching its greatest diminution in about 5 minutes after cessation of exercise.

The very low or negative figures for the minute volume sometimes obtained after exercise indicate probably an increased stroke volume during exercise, which persists for more than 5 minutes after the cessation of exercise.

BIBLIOGRAPHY

BARDEEN. 1918. Amer. Journ. Anat., xxiii, 423.

BRUNS. 1921. Münch. med. Wochenschr., xviii, 907.

BOIGEY. 1921. Presse Med., 17.

BORDET. 1923. Arch. d. Mal. du Coeur, xvi, 118.

BENEDICT AND CATHCART. 1913. Carnegie Inst. of Wash. Publ. no. 187.

COLLETT AND LILJESTRAND. 1924. Skand. Arch. f. Physiol., xlv, 17.

DE LA CAMP. 1903–04. Zeitschr. f. k. Med., li, 1.

DIETLEN AND MORITZ. 1908. Münch. med. Wochenschr., xv, 489.

HENDERSON AND HAGGARD. 1922. Arch. Néerl de Physiol., vii, 378.
1925. This Journal, lxxiii, 193.

HODGES AND EYSTER. 1924. Amer. Journ. Roent. (new series) xii, 252.

HODGES. 1924. Amer. Journ. Roent. and Radium. Therap., xi, 466.

HOFFMAN. 1902. Verhandlund. d. Kong. f. Innere Med., xx, 307.

HOOKER AND EYSTER. 1914. This Journal, xxxv, 73.

HOWELL AND DONALDSON. 1884. Philos. Trans., 139.

KIENBOCK, SELIG AND BECK. 1907. Münch. med. Wochenschr., liv, 1427.

LENNHOFF AND LEVY-DORN. 1905. Deutsch. med. Wochenschr., xxxi, 869.

LEVI. 1906–07. Fortsch. a. d. Geb. d. Roentgenstrah, x, 4.

MEEK. 1924. This Journal, lxxxii, 174.

MEEK AND EYSTER. 1923. This Journal, lxiii, 400.

MENDEL AND SELIG. 1907. Wien. med. Wochenschr., cxi, 135.
MORITZ. 1902. Münch. med. Wochenschr., xlix, 826.
1908. Münch. med. Wochenschr., lv, 713.
NICOLAI AND ZUNTZ. 1914. Berl. klin. Wochenschr., li, 821.
RAAB. 1909. Münch. med. Wochenschr., lvi, 555.
SAVAGE. 1911. Amer. P. E. Review, xvi, 144.
SCHOTT. 1908. Münch. med. Wochenschr., 952, 1538.
SEVRINGHAUS. 1927. This Journal, lxxx, 347.
WHITE. 1924. This Journal, lxix, 410.
WILLIAMSON. 1915. Amer. Journ. Med. Sci., cxlix, 492.

THE ALL-OR-NONE PRINCIPLE AT THE STIMULATED POINT OF NORMAL AND NARCOTISED NERVE

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Received for publication September 21, 1927

Since Bowditch's discovery (1871) of the all-or-none law in heart muscle, the validity of this law has been extended to nerve and other muscle fibres. However, its validity for the nerve fibre is not so firmly established as appears at first sight. The reasons are as follows:

1. In the experiment upon which many physiologists[1] base the validity of this law, the strength of the nervous impulse is measured in terms of its ability to travel through a decremental (narcotised) region. However it was reported by us (Kato, 1924) that the strength of the impulse has no influence at all on its ability to pass a narcotised region; in other words, impulses of varying intensities are extinguished at the same stage of narcosis. Consequently evidence of this kind is inadequate to establish the validity of the law.

2. Previous observations have all been made, not at the stimulated point *A* (see fig. 1), but at some distance (*B*, *C*, or *D*) from this point, taking the muscle contraction or the action current as an indicator. As far as we know, no attempt has ever been made to determine whether or not the size of the response at the stimulated point *A* is dependent on the strength of the stimulus. This question is, indeed, the fundamental criterion of the all-or-none law in its genuine sense. Recently Ishikawa (1927) suggested that nerve belongs to a "system"which does not follow the all-or-none law at the stimulated point (*A* in fig. 1), but the response has the all or none relation if tested at some distance (*B*, *C*, or *D*) from the stimulated point. He defined such a system as an "atypical heterobolic system." Based on the supposed existence of this atypical heterobolic system, he modified Verworn's theory of decrement and published a neo-decrement hypothesis. But he gave no experiment to prove its existence.

Nevertheless, it is not without interest to test the validity of the law at the stimulated point, because we can thus bring Ishikawa's supposition to an experimental test on one hand, and Verworn's theory of heterobolism on the other hand, if we extend the experiment to narcotised nerve. Verworn

[1] Symes and Veley, Verworn, Adrian and others.

(1914) and later Adrian and Forbes (1922)[2] advanced the opinion that the narcotised nerve does not follow the all-or-none law, although the normal one does, i.e., to use Verworn's expression, the normal nerve which is isobol becomes heterobol by narcotisation. The criticism of the experiments of these authors made by Kato (1924, 1926) will be found in his monographs. Moreover, since Verworn's heterobolism is, as is well known, inseparably connected with his theory of decremental conduction in narcotised nerve (Verworn, 1914), we can criticise his theory of decrement by examining the validity of the all-or-none law at the stimulated point of narcotised nerve.

THEORETICAL. There is only one method by which to approach this question: that is the determination of "the absolute refractory period" of the stimulated point A of nerve due to the response evoked by varying strength of the stimulus applied at A. The nerve, if excited by a stimulus, returns gradually to its resting condition. In this case, let us express by "absolute refractory period" the least time required to recover from the first stimulus just enough to respond again to a second stimulus. If the length of the absolute refractory period is not dependent on the strength of the stimulus, then it is certain that the all-or-none law is valid at the stimulated point of nerve. The reasoning is as follows: Suppose that 100 units of energy are available at the point A in its resting condition. If the stimulated point A follows the all-or-none law, then it will discharge, if it responds at all, all the energy available at the moment independently of the strength of the stimulus applied, and will start to recover, unit by unit, from the same zero-stage. The absolute refractory period, that is, the least time required to recover just enough to respond again must, therefore, be equal regardless of the strength of the stimulus. On the contrary, if it does not follow this law, i.e., if heterobolic, then it will discharge different units of the energy according to the strength of the stimulus, say 60 units by a weak and 90 units by a stronger stimulus. It would then start to recover from different stages in the two cases because the point A would have 40 units of the residual energy after the weak and only 10 units after stronger stimulus. Consequently the absolute refractory period as above defined would not exist at all or, if it did exist, could not be the same with different strengths of the stimulus. Thus the question becomes very simple. We have only to examine whether or not the absolute refractory period at the stimulated point is the same with different strengths of the stimulus.

In what way can the absolute refractory period of the stimulated point A be measured? We can answer this question by determining the least interval for muscular summation applying two stimuli successively at the point A. It must, however, be noted that the least interval thus determined is not necessarily the refractory period of the stimulated point A, because in its determination the summated contraction of the muscle at some distance from

[2] Forbes (1926) abandoned his opinion in the paper in which he reported the non-decremental conduction in narcotised nerve.

TABLE 1

Room temperature 21°C.

I. STIMULUS	LEAST INTERVAL (SECOND)					
	A		B		C	
ohms	(−)	(+)	(−)	(+)	(−)	(+)
100	0.00125–0.00131		0.00266–0.00275		0.00368–0.00375	
90	0.00125–0.00131					
80	0.00125–0.00131		0.00266–0.00275		0.00368–0.00375	
70	0.00125–0.00131					
60	0.00125–0.00131		0.00266–0.00275		0.00368–0.00375	
50	0.00125–0.00131					
40	0.00125–0.00131		0.00266–0.00275		0.00368–0.00375	
30	0.00139–0.00148					
20	0.00148–0.00155					

A = normal.
B = at 30th minute of narcotisation.
C = at 50th minute of narcotisation.
(−) = not summated.
(+) = just summated.
Narcotic: 2.0 per cent Urethane-Ringer Solution.

TABLE 2

Room temperature 19°C.

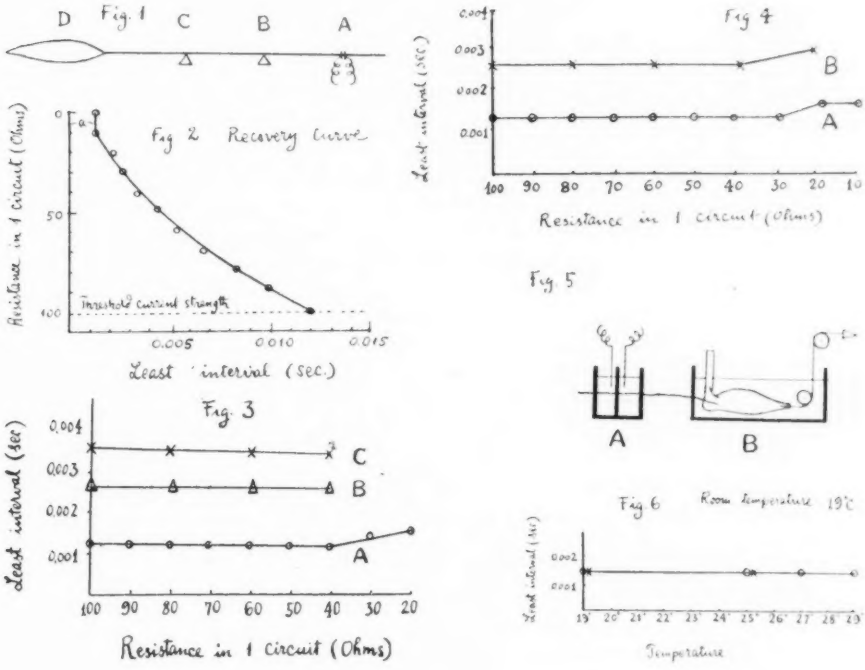
I. STIMULUS	LEAST INTERVAL (SECOND)			
	A		B	
ohms	(−)	(+)	(−)	(+)
100	0.00131–0.00139		0.00250–0.00265	
90	0.00131–0.00139			
80	0.00131–0.00139		0.00250–0.00265	
70	0.00131–0.00139			
60	0.00131–0.00139		0.00250–0.00265	
50	0.00131–0.00139			
40	0.00131–0.00139		0.00250–0.00265	
30	0.00131–0.00139			
20	0.00171–0.00179		0.00296–0.00313	
10	0.00171–0.00179			

A = normal.
B = at 40th minute of narcotisation.
(−) = not summated.
(+) = just summated.
Narcotic: 0.5 per cent Cocaine-Ringer Solution.

the stimulated point is used as the indicator. The following points must, therefore, be proved by control experiments: 1, that the least interval thus determined is not that of the muscle (or neuro-muscular junction), but that

of the nerve itself. When it is so, then 2, that it is actually the least interval of the stimulated point A and not due to other factors during propagation of the impulse to the recording muscle. When it is so, then 3, that it is the absolute refractory period of the stimulated point A.

PRELIMINARY EXPERIMENT. The least time for muscular summation varies, as is well known, according to the strength of the second stimulus. Therefore, the first step in the experiment is to determine the strength of the second stimulus. The sciatic gastrocnemius preparation, taken from



the Japanese toad, is mounted in a moist chamber and the "recovery curve" is obtained with the Lucas pendulum (see fig. 2), applying two successive stimuli at the point A of the nerve through the same fluid electrode. The first stimulus is fixed at the strength which is somewhat stronger than the just maximal one. The strength of the second stimulus is varied by changing the resistance inserted in the primary circuit, the coil distance being fixed to give the threshold strength when there are 100 ohms in the primary circuit. It will be seen that the least interval becomes gradually shorter as the second stimulus is strengthened, till it reaches a constant value (*a* in

fig. 2) when the resistance in the primary circuit is less than 10 ohms; in other words, when the second stimulus is stronger than 10 times as strong as the threshold stimulus. For simplicity we will call the constant value a, obtained by this strength of the second stimulus, "least interval" hereafter.

MAIN EXPERIMENT. One example from twenty-two experiments will be described (see table 1 and fig. 3). The strength of the *first* stimulus is varied by changing the resistance in the primary circuit, the coil distance being so fixed that it gives the just maximal strength when there are 100 ohms in the primary circuit. The second stimulus is fixed at the strength above mentioned to obtain the constant least interval, a. The least intervals are determined at the point A with various strengths of the first stimulus, two stimuli being applied through the same fluid electrode filled with Ringer's solution. A in table 1 (plotted in *A* in fig. 3) shows the result thus obtained at room temperature[3] of 21°C. Then the Ringer's solution in the fluid electrode is replaced by 2.0 per cent urethane-Ringer solution in order to narcotise the stimulating point A. At various stages of narcosis the least intervals are determined as before. Of course in this case the coil distance is readjusted as the strength of the threshold and of the just maximal stimulus changes in narcosis. *B* in table 1 and in figure 3 shows the result obtained after thirty minutes of narcotisation. *C* shows the same after 50 minutes. It will be seen in A (normal nerve) that the least interval remains constant although the resistance is varied from 100 to 40 ohms, i.e., although the strength of the first stimulus is strengthened from the just maximal to 2.5 times as strong. The increased interval in case of 30–20 ohms is nothing other than the "local effect" described elsewhere (Kato, 1926) due to the local injury of the stimulated point by the strong stimulating current. The presence of this local effect alone is nearly sufficient to know that the least interval given above is that of the stimulated point itself. *B* and *C* (both narcotised nerve) show a similar result, although the least interval is increased as the narcosis proceeds. The increase of the least interval in narcosis is due entirely to the slowing of the recovery process and not to decrement. Since this point was conclusively discussed elsewhere (Kato, 1926) we will not go into a detailed description. The other experiments gave similar results without exception. Figure 4 (see table 2) shows the results when 0.5 per cent cocaine-Ringer solution is used as a narcotic.

From these experimental results we can conclude with confidence that the least interval remains unchanged with different strengths of the first stimulus, provided the local effect of the stimulating current does not play a part. And this is likewise true in the narcotised nerve. There is no qualitative difference between normal and narcotised nerve. Moreover, from

[3] All the experiments were made between 17°–22°C.

the presence of the local effect, it is highly probable that the least interval given above indicates the absolute refractory period at the stimulated point. Therefore the validity of the all-or-none law at the stimulated point seems to be established.

According to Lucas (1911) who experimented at low temperature (2°–4°C.) there is 20 per cent difference between the least interval determined with the muscular summation as the indicator and that determined by means of the second electric response of nerve; in other words, the early second impulse traveling down the nerve is cut down between nerve and muscle and is unable to provoke summated contraction. Therefore the least interval obtained in the main experiment with muscular summation as the indicator might be the value modified by the muscle or by the neuro-muscular junction and not be of purely nervous origin. To control this point an experiment was conducted as follows: The experimental arrangement is shown diagrammatically in figure 5. *A* is the same fluid electrode used in the main experiment. *B* contains Ringer's solution at room temperature in which the muscle is immersed. At first the least interval was determined as in the preliminary experiment when the whole preparation was at the room temperature; then the Ringer's solution in *B*, immersing the muscle, was warmed to a desired degree, whereas the nerve outside *B* was kept at room temperature throughout the experiment. At various temperatures the determination of the least interval was repeated to see whether or not this value decreases as the muscle is warmed. From eighteen experiments, all of which were made at a room temperature above 15°C. one example is shown in figure 6. In this case the determinations were made at 19° (room temperature), 25°, 27° and 29° in ascending order (marked with circle) and then again at 25° and 19°C., in descending order (marked with cross), the Ringer's solution being cooled again to room temperature. It will be seen that the least interval remains unchanged (always 0.00148 second), although the temperature of the muscle (and of the neuro-muscular junction) was raised from 19° to 29°C. The other experiments showed similar results. If there are any early second impulses which travel down the nerve but are unable to provoke muscular summation, then the least interval should become shorter when the muscle is warmed, because its recovery process becomes quicker by warming. The experimental result shows, however, that the least interval determined when the whole preparation is above 15°C. at least[4] is not influenced at all by the muscle. Consequently the least intervals obtained in the main experiment, all of which were made above 17°C. are the least intervals of the nerve, and are not modified by the muscle. Moreover, from the presence of the local effect it is clear that they are the least intervals of the stimulated point itself.

[4] The room temperature was 15°C. at the lowest in May. Therefore no experiment was made at the lower temperature.

Therefore, there remains only one point to be considered, i.e., whether or not the least interval obtained in the main experiment is the absolute refractory period of the stimulated point. From the works of Forbes, Ray and Griffith (1923) and of Gasser and Erlanger (1925) it will be easily understood that this least interval is nothing other than the least time required to recover from the first stimulus just enough to respond to the second stimulus. They found that the second impulse once started, increased gradually in size during its propagation, finding the nerve more and more recovered as it travels, because the rate of conduction of the subnormal impulse is less than that of the normal one. In other words the earliest second impulse, if once evoked, can not fail to reach the muscle to provoke muscular summation. Therefore the least interval mentioned above is no other than the absolute refractory period of the stimulated point.

Further, we have experimentally proved that, after the first stimulus, no second impulse can be evoked by the stimulus applied within the least interval. We know this from the experimental fact that the second stimulus applied within the least interval leaves no refractory period, whereas a refractory period is left if the stimulus is applied at or outside the least interval. It was previously reported (Kato, 1926) that the subnormal impulse, however small, leaves, if once set up, a refractory period of the same length as the normal one, independent of whether it reaches the muscle or not. The detailed description of the experiments will be omitted in this paper, because the result of the American authors is sufficient to establish for the present purpose that the least interval we obtained is the absolute refractory period of the stimulated point.

Thus we come to the conclusion that the response at the very point of stimulation follows the all-or-none law in the normal as well as in the narcotised nerve. Consequently neither Verworn's heterobolism nor Ishikawa's "atypical heterobolic system" is valid for nerve. And since the decrement theory is based on heterobolism, it is disproved. On the contrary the theory of decrementless conduction (Kato, 1924; Forbes and his collaborators, 1926) which is based on the validity of the all-or-none law is supported by these experiments.

SUMMARY

Heretofore attempts to establish the validity of the all-or-none law for nerve fibers have studied the response at a distance from the point stimulated. In the present study we have investigated the question at the point of stimulation using therefore the absolute refractory period left by different strengths of stimuli as a criterion and have obtained the following results:

1. In normal as well as narcotised nerve the response at the stimulated point follows the all-or-none law. The difference between the two is only quantitative. This result supports the theory of decrementless conduction which is based on the validity of this law for all conditions of the nerve fiber.

2. Verworn's concept that normal nerve is "isobol" and conducts without decrement, but becomes "heterobol" when narcotised and conducts with decrement is disproved.

3. Ishikawa's "atypical heterobolic system," a system which is "heterobol" at the point of stimulation although the response is "isobolic" if tested at some distance from this point, is not valid for nerve. The nerve is "isobolic" at the point of stimulation. Consequently his neo-decrement hypothesis based on an atypical heterobolic system is erroneous.

BIBLIOGRAPHY

ADRIAN, E. D. AND A. FORBES. 1922. Journ. Physiol., lvi, 301.

BOWDITCH. 1871. Ber. d. säch. Ges. d. Wiss., math.-phys. Kl., 684.

FORBES, A., L. H. RAY AND F. R. GRIFFITH. 1923. This Journal, lxvi, 553.

FORBES, A., H. DAVIS, D. BRUNSWICK AND A. McH. HOPKINS. 1926. This Journal, lxxvi, 448.

GASSER, H. S. AND J. ERLANGER. 1925. This Journal, lxxiii, 613.

ISHIKAWA, H. 1927. Journ. Biophysics, ii, 82.

KATO, G. 1924. The theory of decrementless conduction in narcotised region of nerve. Nankodo, Tokyo.

1926. The further studies on decrementless conduction. Nankodo, Tokyo.

LUCAS, K. 1911. Journ. Physiol., xliii, 46.

VERWORN, M. 1914. Erregung und Lähmung. Jena.

THE RESPIRATORY EXCHANGE OF DIABETIC AND NON-DIABETIC DOGS FOLLOWING HEPATECTOMY

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Received for publication September 24, 1927

Much of the evidence that sugar cannot be formed from fatty acid in the animal organism has been drawn from observations on depancreatised dogs. From this evidence, which is much too extensive to discuss in detail here, two principal deductions have been drawn: 1, that such animals have lost their power to oxidise glucose; 2, that the diabetic R.Q. of 0.69 indicates the exclusive and direct combustion of fat without any of this passing through a carbohydrate stage. To provide further evidence on these points, a series of determinations has been carried out on the respiratory metabolism of hepatectomised dogs, some of which had also been depancreatised two days before hepatectomy. If the above deductions be incorrect, and on the contrary, the R.Q. represents the algebraic sum of two quotients, *a*, the formation of sugar from fat and protein and *b*, the oxidation of this sugar, then hepatectomy should raise the respiratory quotient to nearly unity and there should be no great difference in the R.Q. of diabetic, as compared with non-diabetic preparations. Such reasoning assumes that the liver is the chief organ in which glyconeogenesis can occur.

Methods. The respiratory observations were made in a carefully controlled respiratory cabinet of the Benedict type, the details of which will be published later. Frequent control analyses by burning ether revealed that the instrument was technically accurate. Any movements made by the animal while in the cabinet were graphically recorded. Hourly observations were made, the circuits being changed without interruption. In the first series of experiments *functional* hepatectomy was performed: Under ether anesthesia the hepatic artery was rapidly isolated and ligated. It was then dissected out to its two terminal branches in the portal fissure, which were tied. As an added precaution the gastro-duodenal artery was tied.[2] A straight Eck fistula was then rapidly performed, using for this

[1] The expenses of this investigation were in part defrayed by a grant from the Carnegie Corporation.

[2] We have found that unless these precautions are taken, the blood sugar takes many hours to fall, indicating that some blood is getting to the liver via the anastomotic branches of the right gastro-epiploic and the superior pancreatico-duodenal arteries.

purpose Mann's modification (personal communication) of the method of Fischler and Schroeder (1909). As a substitute for a straight Eck fistula in some of the experiments the portal circulation was disposed of by evisceration. The abdominal incision was closed in three layers. The animal almost invariably made a splendid recovery.

Although these animals were in splendid condition for a number of hours, the onset of hypoglycemia was considerably slower than in surgically hepatectomised preparations. The symptoms of hypoglycemia were atypical, but prompt recovery occurred on giving glucose intravenously. The animals rarely lived long enough to require a second injection of glucose. These differences between functionally and surgically hepatectomised dogs are probably due to a slight circulation of blood through the liver *via* the capsular veins of the diaphragm.[3]

To excise the liver of a dog without disturbing any other viscus, the only method at present is the three stage operation devised by Mann (1921). It consists in the development of a collateral circulation for the inferior vena cava and the portal vein. A reverse Eck fistula is performed, followed in three or more weeks by ligation of the portal vein. At a subsequent operation the liver is removed with the portion of vena cava running through it.

To study the effect of removing the pancreas and liver simultaneously, the following method was adopted (Mann, 1925). The vena cava just below the liver was subtotally ligated with tape, so that about five-sixths of its lumen was occluded. This established a collateral circulation for the inferior vena cava. About 10 days later, the liver and gastro-intestinal tract were removed *en masse*, after ligating the esophagus, rectum, coeliac axis, superior and inferior mesenterics, the vena cava above and below the liver, and any small vessels encountered along the root of the mesentery. Intra-abdominal pressure was maintained by packing with moist towels. The animal almost always made a splendid recovery walking about the room, responding to call and wagging its tail.

Banting and Gairns (1924) showed that the blood sugar of a dog under ether anesthesia began to rise immediately the pancreas was removed. The surgically hepatectomised, eviscerated preparation is accordingly a mildly diabetic organism, the extent of the diabetes increasing with increasing duration of the survival period. The respiratory metabolism of such a preparation probably approximates closely the metabolism of isolated skeletal muscle.

EXPERIMENTAL FINDINGS. These are presented in the following ten protocols. Protocols 1 and 2 concern a surgically hepatectomised non-

[3] It has been shown that the administration of dihydroxyacetone to such a preparation causes a rise in blood sugar, which is not the case in the surgically hepatectomized preparations (Markowitz and Campbell, 1927).

diabetic and diabetic dog respectively, the liver having been removed by the 3-stage operation of Mann. Protocols 3, 4 and 5 concern the findings in three eviscerated surgically hepatectomised dogs. Protocols 6 to 10 present the findings in five *functionally* hepatectomised dogs, one of which was also depancreatised (eviscerated) at the time of hepatectomy, and two of which were depancreatised two days before hepatectomy.

Protocol 1. Irish terrier.
April 5, 1926. Reverse Eck fistula.
May 17, 1926. Weight 7.8 kilo.
9:30. Ether.
9:40. Hepatectomy.
9:55. Operation over.
10:10. Excellent recovery. Runs round room, responds to call, wags tail, etc.
10:15. Blood sugar 0.074 per cent.
10:30. Put in respiratory cabinet.

TABLE 1

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
11:00–12:00	3071	5.24	2667	0.869	2.00	Quiet. Unable to move because of hypoglycemia
	Taken out. Convulsions. Blood sugar 0.052 per cent. 6 grams glucose intravenously. 8 grams subcutaneously. Recovery					
12:50– 1:50	3207	6.24	3176	0.990	2.14	Sleeping or very quiet
1:50– 2:50	3373	6.54	3329	0.987	2.25	Very quiet. Occasional small movement
2:50– 3:50	3676	6.76	3441	0.936	2.43	Standing up—otherwise quiet
3:50– 4:50	3538	6.27	3191	0.902	2.32	Lying down

12:00. Unconscious. Typical hypoglycemic convulsions. 6 grams glucose in 20 per cent solution injected intravenously. Immediate and complete recovery. Walked, responded to call, wagged tail, etc. Eight grams glucose in 20 per cent solution injected subcutaneously.
4:50. Taken out. Animal walked.
6:00. Blood sugar 0.100 per cent. Good condition. Destroyed. About 40 cc. blood in abdomen.
The findings are presented in table 1.

Protocol 2. Hound.
June 26, 1926. Reverse Eck fistula.
July 24, 1926. Ligation of portal vein and pancreatectomy.
July 26, 1926. Weight 12 kilos.
9:00. Ether.
9:22. Hepatectomy completed.
9:40. Animal running around room in excellent condition. Responds to call, wags tail, etc. Put in respiratory cabinet.

2:05. Typical convulsions. Blood sugar 0.054 per cent; 20 cc. 40 per cent glucose intravenously. Immediate recovery. Returned to cabinet.
5:30. Removed from cabinet. Unconscious. Blood sugar = 0.042 per cent. Died while blood sugar was being determined. At autopsy about 100 cc. blood in abdomen.

The findings are presented in table 2.

Protocol 3. Hound.
July 2, 1926. Subtotal ligation of vena cava below liver.
July 6, 1926.
10:00. Ether.
10:30. Complete extirpation of all abdominal viscera including liver.
10:40. Operation completed.
11:10. Walking. Blood sugar = 0.134 per cent. Weight 10 kilos.
11:40. Put in respiratory cabinet.

TABLE 2

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
10:00–11:00	6466	10.89	5543	0.857	2.65	Very quiet
11:00–12:00	6376	11.10	5650	0.886	2.63	Changed position. Very quiet. Splendid condition
12:00– 1:00	5524	9.625	4899	0.887	2.28	Changed position. Very quiet
1:00– 2:00	4999	8.40	4276	0.855	2.05	Changed position. Sleeping. Convulsions
	Taken out. Blood sugar 0.054 per cent. 20 cc. 40 per cent glucose intravenously. Recovery					
2:35– 3:35	4157	6.79	3456	0.831	1.70	Lying down—awake
3:35– 4:35	4128	6.90	3512	0.851	1.63	Awake. Lying down. Unconscious. Blood sugar at 5.30 = 0.042 per cent

2:15. Typical convulsions. Unconscious. Injected 8 grams glucose intravenously and 8 grams subcutaneously. Immediate and complete recovery.
5:45. Died. At autopsy nothing found to vitiate this experiment. Very little hemorrhage.

Findings are presented in table 3.

Protocol 4. Hound.
July 2, 1926. Subtotal ligation of vena cava below liver.
July 12, 1926. Complete extirpation of all abdominal viscera including liver.
9:40. Ether.
10:30. Operation over. Animal fully conscious and walking. Weight 14.4 kilos.
11:15. Put in respiratory cabinet.
12:40. Convulsions. Seven grams glucose intravenously. Complete recovery. Returned to cabinet.

5:30. Twitching.
5:30. Typical convulsions. Ten grams glucose intravenously. Immediate recovery. Returned to cabinet.
7:40. Removed from cabinet in good condition.
8:00. Quite conscious. Walked with staggering gait.
9:10. Convulsions. Six grams glucose intravenously. Complete recovery.

July 13, 1926.
8:00. Found dead. At autopsy no blood found in peritoneal cavity.

TABLE 3

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
12:15–1:15	4136	8.035	4090	0.990	2.09	Moving a good deal. Very restless
1:15–2:15	3387	6.12	3115	0.920	1.69	Sleeping. Typical convulsions
	Taken out. Convulsions. 8 grams glucose intravenously. 8 grams subcutaneously. Complete recovery					
3:05–4:05	3246	5.98	3044	0.938	1.62	Standing up part of the time
4:05–5:05	3644	7.15	3644	1.00	1.84	Very restless. Moving around a good deal

TABLE 4

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
11:40–12:40	6739	11.995	6105	0.906	2.33	Very restless. Became unconscious
	Taken out. Convulsions. 7 grams glucose intravenously. Recovery. Returned to cabinet					
1:05– 2:05	5549	9.595	4884	0.880	1.91	Restless
2:05– 3:05	6640	12.11	6164	0.928	2.30	Quiet
3:05– 4:05	5821	10.20	5192	0.892	2.02	Quiet
4:05– 5:05	4883	8.83	4494	0.920	1.69	Slowly became hypoglycemic
	Taken out. Twitching. Definite convulsions at 5:30. Injected 10 grams glucose intravenously. Immediate recovery. Sat up and wanted to walk. Restrained. Returned to cabinet					
5:40– 6:40	4803	7.96	4052	0.844	1.64	Lying quiet. Standing occasionally
6:40– 7:40	4609	7.85	3995	0.867	1.58	Lying quiet

Findings are presented in table 4.

Protocol 5. Wire haired terrier.

July 6, 1926. Subtotal ligation of vena cava below liver.

July 16, 1926.
9:40. Ether.
10:10. Evisceration of all abdominal organs including liver.

10:20. Operation completed. Weight 6.5 kilos.
11:10. Walked about the room. Excellent recovery.
11:15. Put in respiratory cabinet.
3:20. Taken out of respiratory cabinet—cold and hypersensitive. Five grams glucose. Recovery.
6:20. Semi-conscious. Taken out of respiratory cabinet; 7 grams glucose subcutaneously and 3 grams intravenously. Recovery. Returned to cabinet.
9:30. Sleeping. Wakened and removed from cabinet. In good condition.
9:50. Slight twitching.
10:00. Semi-conscious. Muscular twitchings. Given 5 grams glucose intravenously. Immediate and complete recovery. Definitely jaundiced.

July 17, 1926.

12:10 a.m. Died suddenly. No hemorrhage found at autopsy.

Findings are recorded in table 5.

TABLE 5

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
11:35						
11:35–1:35 (2 hrs.)	5818	10.22	5202	0.894	2.23	Sitting up but not much movement
1:35–3:05 (1½ hr.)	2611	4.90	2494	0.955	1.35	Quiet
	Taken out of cabinet at 3:20. Cold and hypersensitive. 3 grams glucose. Immediate recovery. Walked					
3:45–4:45	1555	2.945	1499	0.964	1.20	Sitting up. Looks up on tapping the window pane
4:45–5:45	1638	2.79	1420	0.867	1.24	Sitting up. Looks up on tapping the window pane
5:45–6:15	787	1.375	698	0.887		Semi-conscious
	Taken out of respiratory cabinet. 7 grams glucose subcutaneously and 3 grams intravenously. Immediate recovery					
7:30–8:30	1692	2.84	1446	0.854	1.28	Sitting up
8:30–9:30	1692	3.01	1532	0.905	1.30	Alternately standing up and lying down

Protocol 6. Airedale.

April 23, 1926.

10:07. Ether.
10:20. Abdominal evisceration, with ligation of stump of portal vein and bile duct. Liver left *in situ*. Excellent recovery. Weight 20 kilo.
12:00. Put in respiratory cabinet till 4.30.
4:30. Stupid. Blood sugar 0.068 per cent.
5:00. Barely conscious. Nine grams glucose in 50 cc. saline intravenously. mediate and complete recovery. Walked normally.
5:10. Put in respiratory cabinet till 7.35.
7:35. Stupid. Blood sugar 0.148 per cent; 10 grams glucose intravenously. Returned to cabinet.
9:00. Died. Abdomen contained about 100 cc. bloody fluid.

The findings are presented in table 6.

Protocol 7. White terrier. 9.5 kilos.

April 8, 1926.

10:10. Ether.

10:40. Straight Eck fistula with ligation of hepatic artery and its branches.

11:05. Operation over. Regained consciousness.

11:55. Blood sugar = 0.110 per cent. Splendid condition.

12:20. Put in respiratory cabinet till 4:05.

TABLE 6

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
12:30–1:30	8988	15.87	8078	0.899	2.23	Quiet
1:30–2:30	8759	15.57	7925	0.905	2.17	Very quiet
2:30–3:30	8578	15.12	7696	0.897	2.13	Very quiet
3:30–4:30	9098	16.43	8363	0.919	2.27	Very quiet
	Taken out. Blood sugar 0.068 per cent. Semi-conscious. 9 grams glucose. Complete recovery					
5:35–6:35	6993	11.10	5650	0.808	1.70	Slight movement
6:35–7:35	7471	13.04	6637	0.888	1.85	Restless
	Taken out. Blood sugar 0.148 per cent					

TABLE 7

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
1:05– 2:05	4945	8.69	4423	0.894	2.58	Quiet
2:05– 3:05	4921	8.62	4388	0.892	2.57	Quiet
3:04– 4:05	4959	8.23	4181	0.843	2.57	Moved occasionally
	Taken out. Blood sugar 0.096 per cent					
4:55– 5:55	4514	7.02	3573	0.774	2.30	Quiet
5:55– 6:55	5007	7.57	3853	0.770	2.56	Stood up several times
6:55– 7:55	5287	7.99	4067	0.769	2.70	Very quiet
	Taken out. Stupid. Blood sugar 0.069 per cent. 9 grams glucose. Recovery					
9:30–10:30	4645	7.44	3789	0.818	2.39	Quiet

4:20. Blood sugar 0.096 per cent. Splendid condition.

4:35. Put back into respiratory cabinet till 8.30.

8:30. Stupid. Blood sugar 0.069 per cent; 9 grams glucose injected intravenously. Recovery. Returned to cabinet till 10:30.

10:30. Taken out of cabinet in good condition. Destroyed. No hemorrhage at autopsy.

Findings are presented in table 7.

Protocol 8. Scotch terrier. 9.3 kilos.

April 15, 1926.

10:20. Ether.
10:40. Straight Eck fistula with ligation of hepatic artery and branches.
11:10. Excellent recovery.
12:35. Blood sugar 0.062 per cent.
1:00. Put in respiratory cabinet till 3:30.
3:30. Stupid. Unable to walk.
3:45. Blood sugar 0.053 per cent; 8 grams glucose injected intravenously. Recovery. Able to walk.
4:00. Returned to respiratory cabinet till 6:30.
6:45. Blood sugar 0.044 per cent. Animal semi-conscious.
8:00. Dead. No blood in abdomen.

The findings are presented in table 8.

TABLE 8

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
1:30–2:30	4408	7.94	4041	0.917	2.37	Moving around a little
2:30–3:30	4176	9.07	3599	0.862	2.22	Quiet
	Taken out. Stupid. Unable to walk. Blood sugar 0.053 per cent. 8 grams glucose. Recovery					
4:30–5:30	5057	8.70	4428	0.876	2.69	Very quiet
5:30–6:30	3697	6.02	3064	0.829	1.81	Quiet

TABLE 9

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
11:45–12:45	4636	7.95	4047	0.873	2.80	Restless
12:45– 1:45	4519	8.11	4128	0.913	2.75	Standing up part of time
1:45– 2:45	3640	6.39	3253	0.894	2.20	Asleep

Protocol 9. Irish terrier.

May 10, 1926.

2:00. Pancreatectomy.

May 12, 1926.

9:50. Ether.
10:20. Operation over. (Evisceration with ligation of stump of portal vein and bile duct.) Excellent recovery. Weight 8.2 kilos.
11:00. Blood sugar = 0.431 per cent.
11:15. Put in respiratory cabinet.
3:55. Died. Very little blood in abdomen. Blood sugar (from heart) = 0.137 per cent.

The findings are presented in table 9.

Protocol 10.

April 10, 1926. Depancreatised.

April 12, 1926.

9:45. Blood sugar 0.378 per cent. Ether.

10:30. Operation over. Straight Eck fistula with ligation of hepatic artery and its branches. Liver very fatty.

10:45. Blood sugar 0.465 per cent.

11:00. Excellent recovery.

11:15. Put in respiratory cabinet till 3:45.

3:45. Blood sugar = 0.265 per cent. In good condition.

4:25. Put in respiratory cabinet till 7:45.

8:00. Blood sugar = 0.159 per cent. Good condition.

8:15. Ten grams glucose in 30 cc. saline slowly given intravenously.

8:20. Vomited; stopped breathing.

8:30. Heart still beating. Autopsy—stoma in good condition. Nothing found to vitiate this experiment.

The findings are presented in table 10.

TABLE 10

TIME	O_2 VOLUME	CO_2		R.Q.	CALORIES PER KILO PER HOUR	REMARKS
		Weight	Volume			
	cc.	*grams*	*cc.*			
11:45–12:45	4365	7.05	3588	0.822	2.14	Quiet
12:45– 1:45	4444	7.17	3650	0.821	2.17	Quiet
1:45– 2:45	4454	7.10	3614	0.811	2.18	Quiet
2:45– 3:45	4559	7.27	3700	0.812	2.23	Moving a little
	Taken out. In good condition. Blood sugar 0.265 per cent					
4:45– 6:45	7835	12.68	6454	0.824	1.93	Very quiet
6:45– 7:45	3737	6.09	3100	0.830	1.83	

Discussion. In protocol 1 (see fig. 1), the R.Q. two hours after hepatectomy was 0.869. The heat output was 2 calories per kilo per hour. The animal during this time was hypoglycemic. The administration of 1.75 gram glucose per kilo caused a rapid rise in R.Q. to 0.99. It stayed at this level for two hours, then fell in the next 2 hours to 0.936 and 0.902 respectively. The administration of glucose caused a gradual increase in heat output, which attained its maximum of 2.43 calories per kilo per hour during the third hour. (The animal was standing during part of this period.) At the end of the experiment the blood sugar was 0.1 per cent, the R.Q. 0.902 and the heat output 2.32 calories per kilo per hour.

We have obtained identical results in two other dogs hepatectomised by Mann's 3-stage operation, although the animals were not in such good condition during the experiment.

Using the Tissot spirometer and the Haldane 10 cc. gas analysis burette, Mann (1924) made some careful estimations on the respiratory exchange before and after hepatectomy. He found that immediately after hepatec-

tomy the R.Q. was always higher than before the operation, but that the R.Q. fell as the animals became moribund. The intravenous administration of 1 gram glucose per kilo always caused an increase in the R.Q. and the oxygen consumption. The total heat output, after hepatectomy, was less than that of a normal animal providing the animal was at rest.

It is of course a question as to just what value can be placed on the R.Q. in a liverless dog as an index of the type of metabolism. In this

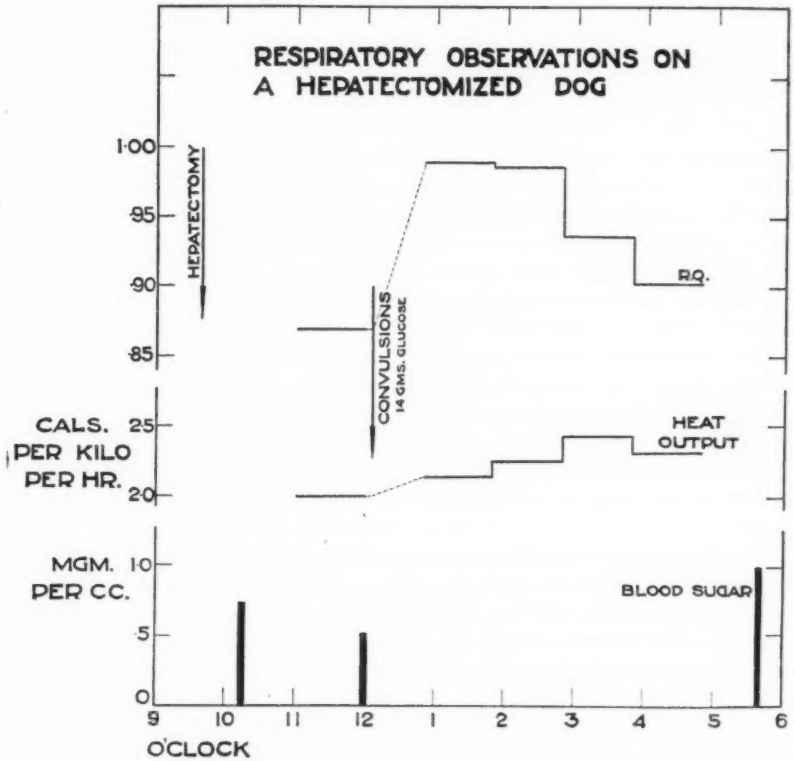

Fig. 1

condition the organism is dying more or less slowly, and the whole course and the end products of metabolism may be altered. The fact that the R.Q. shows the normal behaviour following administration of carbohydrate seems to indicate, however, that this quotient is a more or less reliable guide to the type of metabolism that is going on.

It was shown by Mann (1924) that following hepatectomy the glycogen

content of skeletal muscle showly diminished, so that whereas immediately after hepatectomy the average value was 0.3 per cent, in four hours it had fallen to 0.15 per cent. (The animal had meanwhile received no glucose.) To prevent this diminution of glycogen it was found necessary to inject much larger quantities of glucose intravenously than 0.25 gram per kilo per hour, which is the amount that is merely sufficient to maintain the blood sugar at about its normal level.

With the foregoing considerations in mind, the interpretation of the significance of the R.Q. in a liverless dog becomes impossible until more is known about the behaviour of the metabolism in such a preparation. The following are possible causes of a quotient of 0.87 in it: 1. That more than twice as much carbohydrate as fat is being oxidised (taking the R.Q. at its face value). 2. That carbohydrate is being exclusively oxidised, accompanied by the breakdown of an oxygen-poor into an oxygen-rich substance, a breakdown which is prevented by the presence of a large amount of circulating carbohydrate acting in the presence of insulin. 3. That protein, breaking down into amino acids and other metabolites liberates compounds which can serve as a source of energy, and that the oxidation of these *plus* that of carbohydrate, gives a quotient of about 0.87. This hypothesis would imply that such a breakdown of protein can be spared by carbohydrate acting in the presence of insulin. (The protein sparing action of carbohydrate in the intact non-diabetic organism is in favour of this hypothesis.) 4. Just what part is played by the degradation of an unknown carbohydrate complex in modifying the R.Q. of a liverless preparation is unknown. Much of the evidence today indicates that insulin brings about the formation of a carbohydrate complex in skeletal muscle.

Whatever be the meaning of the R.Q. in our experiment, the heat output is too great to be accounted for by the oxidation of the glycogen which is known to disappear. Thus, assuming the animal's skeletal muscle to constitute 35 per cent of its body weight (Whipple, 1926) and the glycogen of the muscles, 0.3 per cent (Mann, 1925) the amount of glycogen present in our dog immediately after hepatectomy was $\frac{35}{100} \times 7.97 \times 0.3 = 8.4$ grams. In 4 hours (using Mann's findings), this quantity would dwindle to 4.3 grams. The amount of energy that could be derived from oxidation of the glycogen which disappeared is, therefore, $4.2 \times 4 = 16.8$ cals. which is sufficient for only a little over an hour, nor would the addition of the disappearing blood sugar add materially to this supply.

In the case of the diabetic dog that was hepatectomised by Mann's 3-stage operation (protocol 2) the R.Q. averaged 0.87 for 4 hours before the administration of sugar, and it failed to rise as a result of this administration. Assuming that this quotient has the same significance

as in the intact animal, the tissues of a diabetic organism must still retain their powers to oxidise glucose. This conclusion harmonises with the rapid disappearance of blood sugar in the diabetic liverless preparation, and with the prompt relief of the hypoglycemic symptoms by glucose. The failure of the R.Q. to respond to glucose is, therefore, very significant, although its interpretation is obscure. It probably indicates the failure of the sugar to undergo some type of transformation which occurs only in the presence of insulin but this transformation is not a necessary step in the antidoting action of glucose against hypoglycemic convulsions. Other evidence for such a conclusion is the following: 1. The changes in the phosphate content of blood and of urine following administration of glucose, do not occur in the diabetic organism (Bollinger and Hartman, 1925; Markowitz, 1926). 2. The injection of glucose into an eviscerated preparation does not lead to a deposition of glycogen in the muscles unless insulin is also injected (Best, Hoet and Marks, 1926; Choi, 1927).

It will be noted that the animal died of hypoglycemia. It is not likely that it would have lived much longer in any case, as the O_2 consumption was much below normal. We spent much time in an attempt to get other hepatectomised diabetic dogs that would stay in good condition for a large number of hours. In this we failed. Shortly after hepatectomy such animals develop typical convulsions which are promptly relieved by glucose; but the animal collapses again, with the blood sugar above normal. Mann's experience with hepatectomy in diabetic dogs is identical (1925).

The eviscerated hepatectomised dogs in protocols 3, 4 and 5 illustrate that the R.Q. immediately after hepatectomy is 0.9 or more. The animal developed hypoglycemia very rapidly. Following the first injection of glucose for the relief of hypoglycemia there was a slight rise in R.Q. In dogs of protocols 4 and 5 the heat output fell progressively. The animal in protocol 3 was very restless, which probably accounts for the high initial R.Q. of 0.99 and for the irregularity in the heat output. It should be pointed out that since the respiratory observation was not begun for at least 15 minutes after the injection of sugar slight or evanescent rises in the R.Q. may have been missed. Also since each observation lasted one hour a temporary but considerable rise might easily have been masked.

Experiments were made on three other such eviscerated preparations that had been depancreatised two days previously. They are not reported as they only lived about four hours. The R.Q.s during this time varied between 0.87 and 0.92.

Protocols 6, 7 and 8 illustrate the findings in functionally hepatectomised dogs. Protocols 7, and to a lesser extent 8, show the rise in R.Q. which follows administration of glucose to an organism retaining the pancreas, this being absent in the animal of protocol 6.

In protocols 9 and 10 the animals were depancreatised two days before functional hepatectomy. The R.Q.s averaged 0.89 and 0.82 respectively.

As has also been previously shown by Mann, the removal of the liver from diabetic as well as from non-diabetic preparations, is followed by hypoglycemic convulsions which are effectually antidoted by glucose. The hyperglycemia and glycosuria of diabetes are, therefore, dependent on an over-production of sugar by the liver, and not primarily on a diminished power of the tissues to oxidise glucose. The respiratory quotients of diabetic liverless dogs reported by us support this contention, in that before the administration of glucose the quotients of diabetic and non-diabetic liverless dogs are very similar. The work of Burn and Dale (1924) on decapitated eviscerated cats is in agreement with this conclusion, although these authors state that following evisceration the R.Q. is unity. The preparations employed by us were more nearly in a physiological condition, and perhaps this may explain the difference in the findings.

Different workers previously have been unable to demonstrate that diabetic tissues have lost their power to oxidize glucose, the most convincing experiment being that of Mann (1925). When the pancreas and part of the liver were removed from a dog upon whom a preliminary straight Eck fistula had been performed, the animal manifested neither hyperglycemia nor glycosuria.[4]

The fact that the liver is essential for maintaining the normal concentration of sugar in the blood, and indirectly of glycogen in the muscles, emphasises that the transformations of metabolism, especially carbohydrate metabolism, are in a state of dynamic equilibrium. In the intact starving organism the amount of sugar secreted into the blood by the liver in the course of a day is much greater than can possibly come from protein. Such a large quantity of carbohydrate can come only from fat, or some (as yet) unknown compound. The fatty infiltration which constantly occurs when glycogen disappears from the liver suggests that the source of the newly formed blood sugar is fat.

At any rate, no calculation based upon the R.Q. of an intact starving organism is valid unless it considers this function of the liver in glyconeogenesis.

SUMMARY AND CONCLUSIONS

1. After removal of the liver in a dog by Mann's 3-stage operation, the R.Q. was 0.87. Injection of a large dose of glucose raised the R.Q. to 0.99 for 2 hours, after which it slowly fell. Following the injection of glucose there was a slow rise in the heat output which attained a maximum amounting to about 20 per cent more than that before injection. The rise in R.Q following glucose was obtained in two other dogs hepatectomised by Mann's 3-stage operation.

[4] The general condition of the animal was like that of a depancreatised dog.

2. The R.Q. of a liverless dog that had been depancreatised two days previous to hepatectomy by Mann's 3-stage operation averaged 0.87 for a period of 4 hours before hypoglycemia set in. The injection of a large dose of glucose was followed by a fall in the R.Q.

3. The R.Q. of three surgically hepatectomised eviscerated preparations varied between 0.89 and 0.99 before the administration of glucose. The R.Q. showed but little change following the injection of glucose, presumably because of the absence of the pancreas.

4. One eviscerated functionally hepatectomised animal had an R.Q. of 0.9 for four hours before the injection of glucose, which was followed by a slight fall in the R.Q.

In two animals functionally hepatectomised by straight Eck fistula *plus* ligation of the hepatic artery and its branches, administration of glucose caused a rise in the R.Q.

In two functionally hepatectomised animals that were depancreatised two days before hepatectomy, the R.Q.s averaged 0.89 and 0.82 respectively.

5. As judged by the R.Q. following hepatectomy, there is no evidence that the depancreatised animal has lost its ability to oxidise carbohydrate. This conclusion harmonises with that of Deuel, Wilson and Milhorat (1927) in regard to the ability of phlorhidzinized dogs to oxidise glucose. The sugar which appears in the urine of depancreatised dogs is, therefore, not the sole metabolic expression of all the glucose that is produced in the animal's body, since much of the latter is being oxidized. Calculations based upon the extent of the glycosuria in such preparations cannot be safely used as evidence that carbohydrate is not formed from fatty acid in the animal body.

I wish to express my thanks to Prof. J. J. R. Macleod, under whose direction this investigation was conducted.

BIBLIOGRAPHY

BANTING, F. G. AND S. GAIRNS. 1924. This Journal, lxviii, 24.
BEST, C. H., J. P. HOET AND H. P. MARKS. 1926. Proc. Roy. Soc., c, B, 33.
BOLLINGER AND H. P. HARTMANN. 1925. Journ. Biol. Chem., lxiv, 91.
BURN, J. H. AND H. H. DALE. 1924. Journ. Physiol., xlix, 164.
CHOI. This Journal (in press).
DEUEL, H. J., H. E. WILSON AND A. T. MILHORAT. 1927. Journ. Biol. Chem., lxxiv, 265.
FISCHLER, F. AND R. SCHROEDER. 1909. Arch. f. exper. Path. u. Pharm., lxi, 428.
MANN, F. C. 1921. Amer. Journ. Med. Sci., clxi, 37.
1925. Ergebn. d. Physiol., xxiv, 379.
MARKOWITZ, J. AND W. R. CAMPBELL. 1927. This Journal, lxxx, 548.
WHIPPLE, G. H. 1926. This Journal, lxxvi, 693.

THE INTERRELATIONSHIP OF THE CARRIERS OF VITAMINS A AND B AS AFFECTING THE GROWTH AND DEVELOPMENT OF THE TISSUES AND ORGANS OF YOUNG ANIMALS[1]

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Received for publication September 24, 1927

The use of the synthetic ration by nutrition workers has begun to indicate the existence of other dietary factors in addition to those already recognized. While feeding groups of rats with a ration, presumably balanced and adequate, it was observed that young rats failed to grow at a normal rate and that few of the females matured sexually. During this stunted condition the hair on many of these rats began to look oily and later became excessively greasy. If the same ration was fed to adult rats, the lactating mothers and their baby rats developed this greasiness to an extreme degree, and in all cases the hair became thin and showed a tendency to fall. Plate 1, A and B, shows severe cases of greasiness in lactating mother and litter, and in a litter of young rats.

These results were so pronounced that it seemed desirable to inquire into the nature of the greasy secretion of the hair and the nutritive factors which might cause its production: also to study any relationship which might exist between this greasy condition, delayed growth and underdevelopment or inactivity of the sexual organs.

The medical literature describes an excessive greasiness of skin and hair in human beings, called "seborrhea," apparently caused by the hypersecretion of sebum by the sebaceous glands (1). Dermatologists, such as Stelwagon (2), Pusey (3) and Sutton (4), mention seborrhea as occurring on those parts of the body where the largest number of sebaceous glands

[1] Published with the approval of the Director as Paper no. 639, Journal Series, Minnesota Agricultural Experiment Station. The results here presented are taken in large part from a dissertation presented by Jessie E. Richardson in partial fulfilment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1925.

are found. For this reason the scalp and hair are often affected. Adolescence and the time of the climacteric in adult women are also frequently characterized by a distinct increase in sebum. These observations seem to suggest that the sebaceous glands sympathize with changes in the sexual organs.

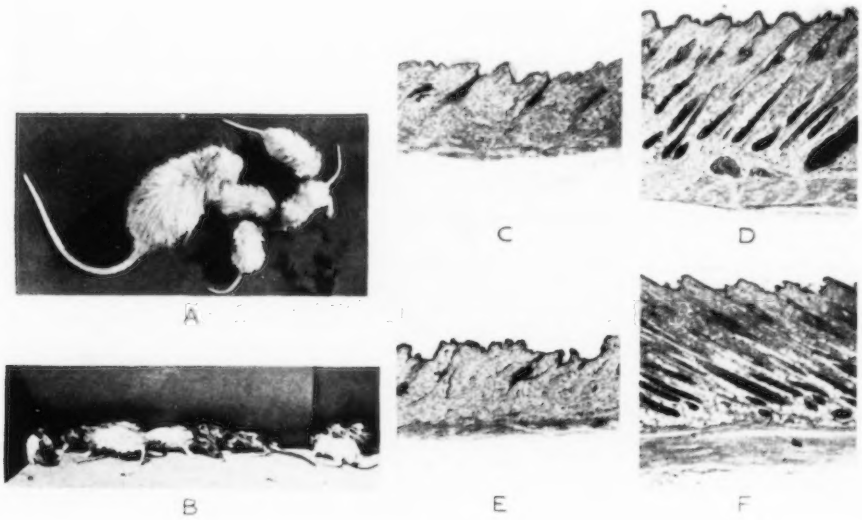

Plate 1. A. Greasy lactating mother and litter.

B. Greasy young rats.

C. Microscopic vertical section of skin from a rat showing the greasy condition. Weight, 74 grams.

D. Vertical section of skin from normal weight control for rat in C. Weight, 79 grams.

E. Vertical section of skin from rat showing greasy condition. Weight, 70 grams, age 8 weeks.

F. Vertical section of skin from normal age control for rat (E). Weight, 125 grams, age 8 weeks.

No definite facts are known regarding the etiology of seborrhea. Dyspepsia, indigestion, constipation and disturbances of the liver and sex organs have been mentioned as contributory if not causative. Other suggestions involve anemia, general debility, heredity, acidosis, catarrh, heat, exercise, alcohol or toxins. Sabouraud (5) contends that seborrhea is parasitic and thus contagious. MacLeod (1) and Scholtz (6) are among the few who refer to the ingestion of fatty foods as a cause of increased

sebum. All investigators recognize the need for further study to determine the actual factors responsible.

Only a few nutrition workers have mentioned the occurrence of excessive oiliness in their experimental subjects. In most cases they have usually attributed it to mechanical contact with oily food and not to any specific dietary deficiency. Meska (7) does report an experiment carried on by Abderhalden in which rats, kept for some time on a diet deficient in vitamins, neglected the care of their bodies and as a result the skin became thin and diseased. Avitaminosis was considered the cause of the condition.

Paul le Gendre (8) specifically mentions seborrhea as being produced by faulty fat metabolism due to a lack of vitamins. He says that there is an insufficiency of combustion so that fat accumulates in the blood causing lipemia. Scholtz (6) speaks of seborrhea as due to the faulty metabolism of milk fat followed by its rapid absorption and deposit in the skin. Frank (9) reports some feeding experiments with rats using a diet containing 80 per cent milk fat. The rate of growth was good but with very few exceptions, the hair was rough, thin and appeared wet. These results were attributed to one-sided vitamin feeding involving an excess of vitamin A and a relative deficiency of vitamins B and C.

It is thus evident that the appearance of excessive oil on the hair and skin of the human being or experimental animal seems to serve as an index to some abnormal internal condition with its possible origin in some sexual, nutritional or metabolic situation.

EXPERIMENTAL. Healthy young rats were chosen as experimental animals. When 28 to 30 days old, weighing about 45 to 50 grams, groups of three rats, or in some cases single rats, were placed in wire-mesh cages with a screen floor three meshes per inch above shavings that prevented the rats from having free access to their feces—a possible source of both known and unknown food factors. The feeding dishes were so constructed that the rats could not make bodily contact with the food. Weekly records of weight and food intake were kept.

The excess of fatty material on the hair of the rats naturally suggested the advisability of first studying the fat portion of the ration. There was no reason to think that the fat was excessive in amount for other nutrition workers have used as much as 24 per cent fat in their rations with no apparent ill effects. The nature or source of the fat was therefore the first point considered.

A mixture of purified food materials, formerly considered adequate in quality and quantity but producing the peculiar results previously mentioned, was adopted as the first basal diet. The composition of this diet is as follows on page 715.

Basal diet I

	per cent
Purified casein[2]	18
Agar	2
Salt mixture (McCollum's 185)	4
Dextrin[3]	61
Fat	15

0.6 gram of dry starch-free yeast[4] was given to each rat daily.

Figure 1 shows the results of various combinations of Crisco and butterfat, Crisco and butter, Crisco and hydrogenated[5] butterfat, and Crisco and cod-liver oil all in comparison with Crisco as the sole fat in the above diet. Group I, the control group, with 15 per cent Crisco in its diet grew very well considering its lack of vitamin A. They were sleek and active but the females showed no signs of sexual maturity, judged by the lack of vaginal orifice.

Groups II, III and IV showed very little improvement in growth over the control group, although butterfat was added to their ration in increasing amounts. At the end of eight weeks, there was a marked falling off in their general appearance. Rats in group II remained almost stationary in weight, groups III and IV showed a marked decline and IV became greasy. These rats looked thin and old and they huddled together as if cold. Their coats were rough, appearing to be a mixture of lanugo and adult hair. None of the females seemed to mature. Some nutritional disturbance was quite evident.

When whole butter replaced the butterfat in groups V, VI, and VII some positive results were obtained. Growth was improved, the rats seemed in good health, and two females reproduced, rat 15 having a litter of 5 and rat 17, a litter of 3. It should be noted that a decline in growth rate again occurred after eight weeks and that groups VI and VII showed almost identical growth, although VI received only 2 per cent butter while VII received 5 per cent (fig. 1).

[2] The casein was prepared from skim milk by the grain-curd method, then purified by the Osborne and Mendel method and finally extracted with hot 80 per cent alcohol and ether, dried and ground to a fine powder.

[3] The dextrin was made by autoclaving tapioca for 3 hours at 15 pounds' pressure, then drying and grinding.

[4] The yeast was obtained from the Northwestern Yeast Company, Chicago.

[5] This was prepared by taking rendered butterfat, adding a trace of nickel salt for a catalytic agent and holding it at a temperature of 240°C. for 2½ hours, while hydrogen from a pressure tank was permitted to bubble slowly through the fat. When the hydrogenated butterfat was cooled, it was a snowy white, with a texture and flavor much like Crisco.

The poor growth obtained when butterfat was used with the Crisco suggested removing vitamin A by hydrogenation. To get rid of this factor might give an opportunity for some other element in the butterfat to show

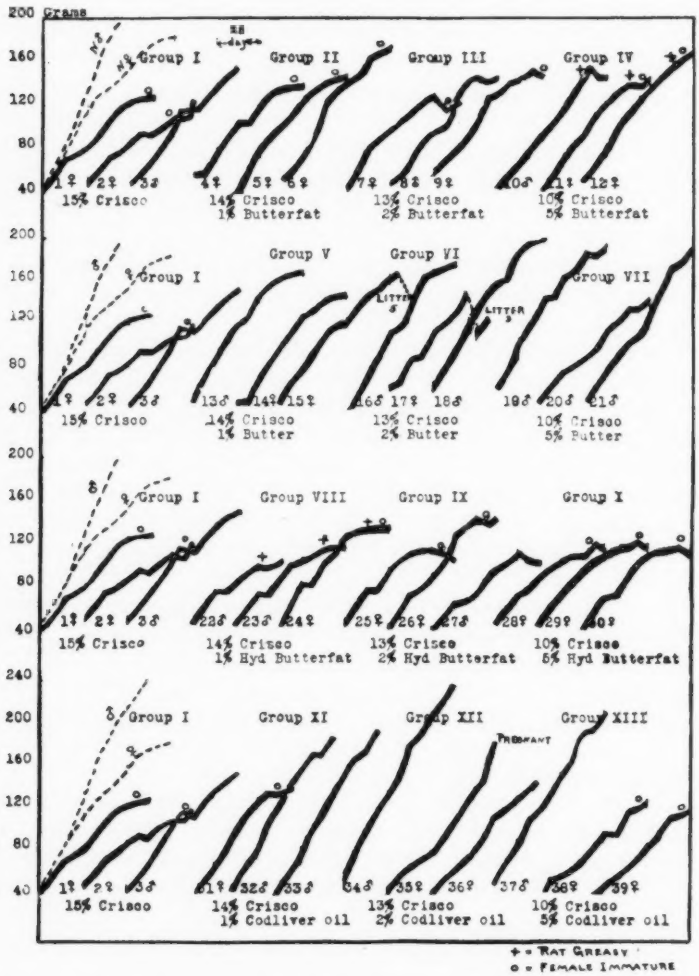

Fig. 1. The growth curves of rats fed on basal diet I where the fat was supplied by a combination of Crisco with butterfat, whole butter, hydrogenated butterfat and cod-liver oil, respectively, and in varying proportions. The normal growth curves represent the average weights of several hundred of the stock rats in this laboratory.

its effect upon growth. The third series accordingly made use of hydrogenated butterfat in combination with Crisco. The food intake was ample but the rats showed little growth from the sixth to the tenth weeks. Group IX appeared better than the others. When autopsied they all were found to have distinct cases of osteoporosis.

In the next series, cod-liver oil, rich in vitamin A[6], was chosen to replace butterfat. In group XI, the males grew very well but the female was stunted and failed to mature. In group XII, the male grew to a normal rate, one female became pregnant, but the other female remained below normal. Group XIII presented some unlooked-for results. With 5 per cent cod-liver oil, the females failed to mature and grew no better than those in the control group with 15 per cent Crisco.

Since rather uniform results were secured from the four series of experiments just described, it served to reëmphasize our previous experience. Again it was indicated that synthetic rations supplied with presumably sufficient protein, minerals and vitamins A and B were unable to induce normal growth and maturity, and at times appeared to be productive of a greasy condition of the skin and hair.

Figure 2 shows the results of a second set of experiments comparing various combinations of Crisco and other fats with Crisco alone or butterfat alone. These experiments differed from those in figure 1 in the use of another source of vitamin B,—the alcoholic extract of ether-extracted wheat embryo. The basal diet was as follows:

Basal diet II

	per cent
Purified casein	18
Agar	2
Salt mixture (McCollum's 185)	3.7
Wheat embryo extract on dextrin	10.3[7]
Dextrin	51
Fat	15

In the first series, groups XIV–XVII, figure 2, Crisco was again combined with butterfat. The growth obtained was almost negligible during a period of seven weeks. The rats changed from their baby appearance and ways to old emaciated animals. The only growth observed was an increase in the length of the skeleton. None of them matured and during the third week they became greasy. A striking resemblance appeared in the growth curves when 15 per cent Crisco and 15 per cent butterfat, respectively, were used.

[6] The cod-liver oil used was vitamin A tested oil for animal feeding obtained from the E. L. Patch Company, Boston.

[7] The equivalent of 15 grams of ether extracted commercial wheat embryo.

In the second series, olive oil replaced the Crisco in combination with butterfat. A slightly different picture was presented. Groups XVIII and XIX were sleek and plump although they failed to grow and mature, but group XX, with 10 per cent butterfat, showed rough fur and became emaciated.

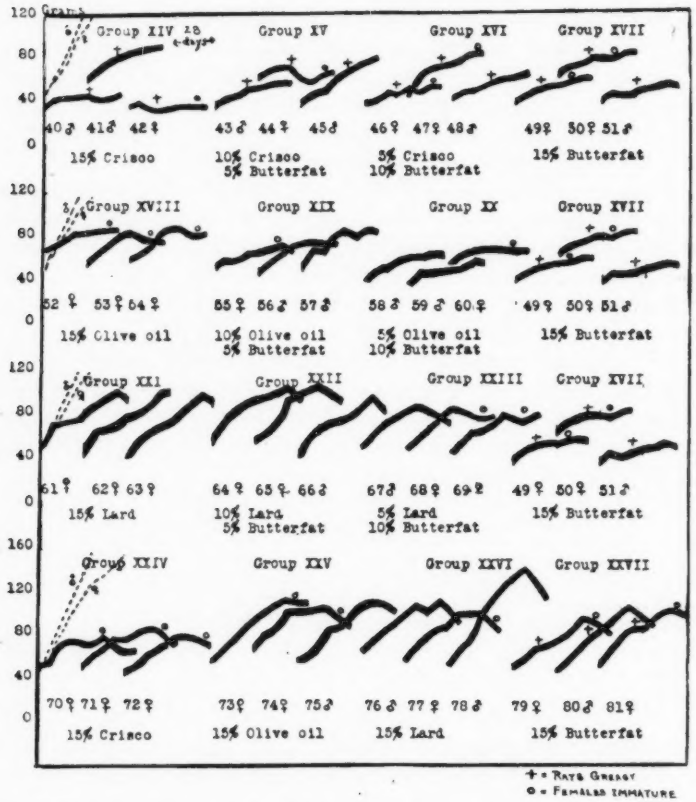

Fig. 2. The growth curves of rats fed on basal diet II, where the fat was supplied by Crisco, olive oil, lard and butterfat, alone and in varying combinations.

The use of lard and butterfat, alone and in combination, in the third series, added more perplexities to the situation. The ration containing 15 per cent lard (group XXI) produced far better rats in general condition and vitality than did 15 per cent butterfat (group XVII). The derogatory effect of butterfat upon lard seemed to appear in their combination.

These results were so contradictory to the prevailing ideas regarding the relative food values of the respective fats,—butterfat, lard, Crisco and olive oil,—as established by previous experiments in nutrition, that it seemed best to repeat some of this work. Four groups were again started on basal diet II. Group XXIV was given 15 per cent Crisco: group XXV, 15 per cent olive oil: group XXVI, 15 per cent lard and group XXVII, 15 per cent butterfat. As the rats in the previous experiments became so

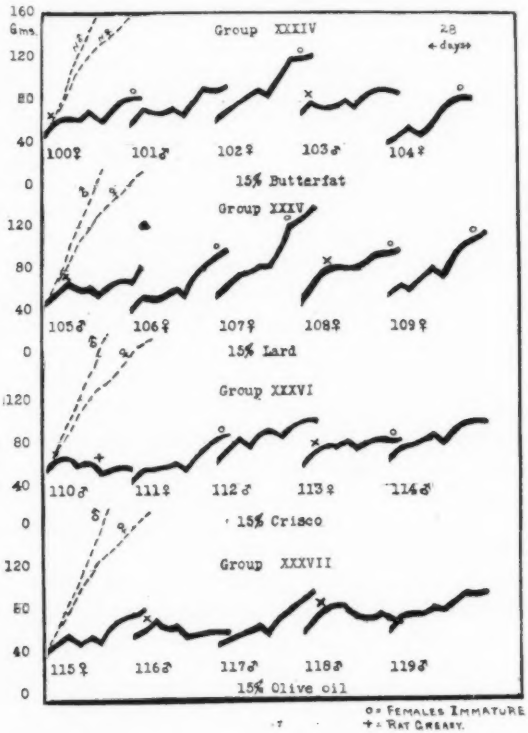

Fig. 3. The growth curves of rats fed on basal diet II, made up with butterfat, lard, Crisco and olive oil, respectively. Where a cross, x, is marked on the curve, 0.8 per cent $CaCO_3$ was added to the ration.

emaciated, one drop of cod-liver oil was given to each rat per day for protection. The results almost duplicated those formerly obtained. The rats receiving Crisco grew very little but appeared well and were active: those on butterfat became greasy during the fourth week, after which they grew very little, were inactive, huddled together and slept most of the time.

The rats fed with lard looked well-kept and grew more than any of the others, although not at a normal rate. Those on olive oil almost equalled in growth and general appearance those fed with lard.

In the next set of experiments shown in figure 3, each rat was placed in an individual cage, thereby reducing both the probabilities of greasiness through bodily contact with one another and ease of securing feces. Group XXXIV was furnished with 15 per cent butterfat: group XXXV, with 15 per cent lard: group XXXVI, with 15 per cent Crisco and group XXXVII, with 15 per cent olive oil. Each rat also received one drop of cod-liver oil per day.

After two weeks, a slight change was made in the salt mixture given to two rats out of each group, as indicated by a cross, x, on the growth curves in figure 3. It had been noted that the bones of the stunted animals were often in a state of osteoporosis. Givens and Mendel (10) have indicated a relationship between the fat and calcium intake and the calcium storage. Hickmans (11) and Holt and Fales (12) each emphasize that the intake of both fat and calcium should be liberal, especially for children. In order to secure a level of 0.65 per cent calcium in a diet containing 3.7 per cent of McCollum's salt mixture 185, it is necessary to add 0.8 per cent calcium carbonate. This was done for rats 100, 103, 105, 108, 110, 113, 116, 118 shown in figure 3.

In these experiments, as in the case of group XXI, figure 2, the rats with lard in their ration showed better growth and state of health than the others. The olive oil, butterfat and Crisco diets produced quite similar results. In all cases where calcium carbonate was added to the food, the rats showed less of an inclination to grow. Rat 110 in the Crisco group, became greasy and died. Evidently calcium bore some relationship to the factor responsible for growth depression.

Figure 4 shows the results of a further test of the effect of additional calcium in the ration. Group XXXVIII had 15 per cent butterfat, 0.8 per cent calcium carbonate in their ration and one drop of cod-liver oil every day. Group XL, lard: group XLII, Crisco: and group XLIV, olive oil with the same additions. Four other groups (XXXIX, XLI, XLIII, XLV) were run in a parallel experiment, with the exception that the cod-liver oil

Fig. 4. The growth curves of rats fed on basal diet II, made with butterfat, lard, Crisco and olive oil, respectively. To half of the groups, cod-liver oil was given for protection, and to the other half, spinach oil.

Fig. 5. The growth curves of rats fed on basal diet II, where the fat was supplied by lard and Crisco, respectively. In groups XXVIII and XXIX, sodium butyrate, salt of butyric acid (a fatty acid peculiar to butterfat) was added to each ration.

Fig. 6. The growth curves of rats fed on basal diet II, when modified to give varying proportions of protein and fat.

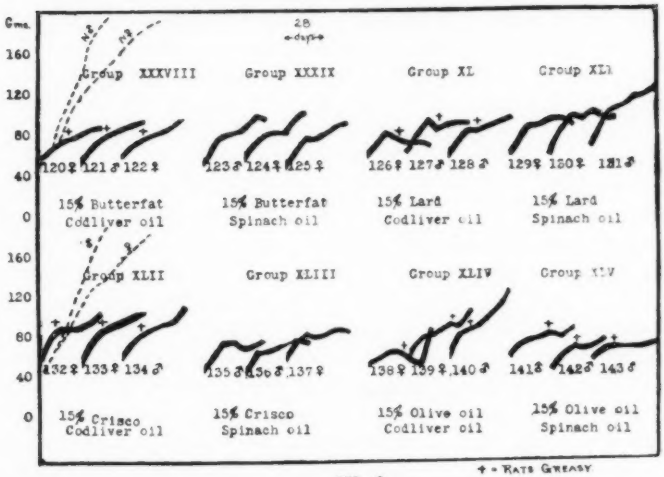

FIG. 4.

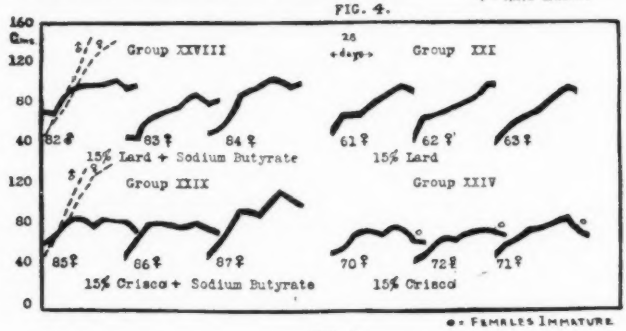

FIG. 5

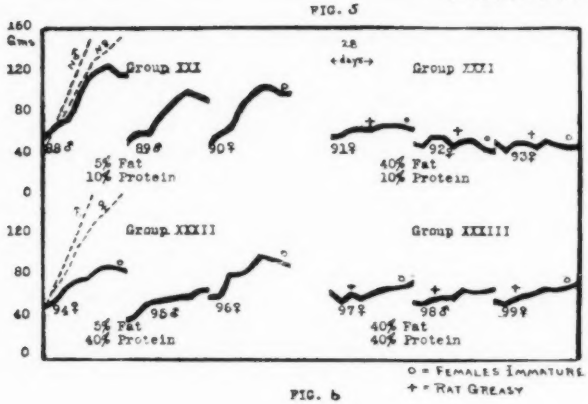

FIG. 6

was replaced by 30 mgm. of spinach oil[8] per day for each rat, in order to supply vitamin A directly from a plant source.

Among the rats receiving the additional calcium carbonate and cod-liver oil, those fed olive oil showed the best growth and general health: those fed Crisco came next: then butterfat: then lard. All these rats were greasy at some time during the experiment. Among the rats given spinach oil in place of the cod-liver oil, those on the lard diet ranked first in general appearance and growth; those on olive oil were greasy and emaciated. It should be noted in the results from the two series of experiments shown in figure 4 that the order of the fats is just reversed in their ability to promote growth under given conditions. This indicates different relationships between cod-liver oil, spinach oil and growth.

Many of the experiments we have conducted in our study of this problem seemed to indicate that butterfat possessed some quality that was depressing to the growth and related to the greasy condition. This suggested a more detailed study of butterfat itself. Comparing its chemical composition with that of the other fats being used, one of the most outstanding differences is the presence of about 3 per cent butyric acid in the butterfat. Thinking that butyric acid might be the offending compound, it was decided to add an appreciable amount of the acid to lard and to Crisco. The direct addition of the acid to a ration was impossible on account of its extreme odor and irritating properties. Instead, 1.12 per cent of sodium butyrate was added to basal diet II. The results are shown in figure 5.

The rats seemed to like the added butyrate, for they ate greedily of their rations, but no difference could be observed in the weight or general appearance of the rats receiving the butyrate (groups XXVIII and XXIX) when compared with the rats in the control groups, XXI and XXIV.

An assertion of Maignon (13), in which he states that fat influences the metabolism of protein, suggested another avenue of attacking the problem. Maignon believes that when there is not a proper proportion of fat to protein, the protein is not utilized and toxic, harmful products result. Acting on this suggestion, four modifications of basal diet II were prepared to furnish 1, low protein and low fat; 2, low protein and high fat; 3, high protein and low fat; and 4, high protein and high fat. The constituents of these diets are given below. In addition, one drop of cod-liver oil was fed to each rat daily. The wheat embryo extract was in each case equivalent to 15 grams of ether extracted embryo.

[8] Fresh green leaves of spinach were dried at a low temperature, ground to a powder and extracted for about 12 hours with warm ether in a Soxhlet apparatus. The brilliant green extract obtained was evaporated on dextrin and fed in small quantities to the rats.

Special diet 1

Low protein and low fat

	per cent
Purified casein	10
Agar	2
Salts (McCollum 185)	3.7
Wheat embryo extract	11
Dextrin	67.8
Crisco	5
Cystine	0.5

Special diet 2

Low protein and high fat

	per cent
Purified casein	10
Agar	2
Salts (McCollum 185)	3.7
Wheat embryo extract	11
Dextrin	32.8
Crisco	40
Cystine	0.5

Special diet 3

High protein and low fat

	per cent
Purified casein	40
Agar	2
Salts (McCollum 185)	3.7
Wheat embryo extract	11
Dextrin	38.3
Crisco	5

Special diet 4

High protein and high fat

	per cent
Purified casein	40
Agar	2
Salts (McCollum 185)	3.7
Wheat embryo extract	11
Dextrin	3.3
Crisco	40

The results of feeding the above diets are shown in figure 6. Good growth was maintained in group XXX for six weeks on the low protein, low fat ration, then the weights declined. Fair growth occurred in group XXXII with high protein and low fat, but in both cases where the fat was high (groups XXXI and XXXIII), whether the protein was high or low, the rats just maintained their weights for eight weeks, at times looking greasy or rough coated. While these results were rather consistent, they offered little help in the problem.

The frequent appearance of greasiness in the experimental animals caused us to wonder if there might be a direct transfer of the fat in the diet to the skin and hair through some faulty metabolism. Methods for testing this transfer have been mentioned in the literature by Read (14) and Gage and Fish (15). They used a fat-soluble dye, Sudan III, which is added at the rate of 0.1 gram of dye for every 10 grams of fat. The dye seems to adhere to the fatty acid portion of the fat molecule and is not transferred during digestion, absorption or assimilation of the fat. We adopted this method and added Sudan III to the fat in our rations, making them a brilliant red. Great care was observed in feeding this colored ration to the rats. They were fed separately, three times a day and when they had finished eating, their mouths and front paws were washed to prevent the spreading of any dye on the outside.

Within five hours the ears and eyes of the albino rats took on a deeper pink color and in twelve hours there was a decided purplish tint to the skin that showed through the white fur. Where the rats were greasy, there appeared in the fur itself a dirty yellowish-pink color, but so faint that when hairs were examined under the microscope, no color could be

seen in the canal. When these rats were autopsied, the viscera were brilliant with red color where the fat had been deposited. Read (14) mentions that the Sudan III is excreted in the feces but not in the urine of normal animals. In our animals, the urine was very pink in color while no trace of Sudan III was noticed in the feces.

Since greasiness on the hair of the rats might serve as an index of abnormal activity in the connecting organ,—the skin, a microscopic study was made of cross-sections of the skins of greasy rats and also of the skins of a normal weight control rat and a normal age control rat. A very marked degeneration is to be noted in both cases where the rats were greasy. These are shown in plate 1, C, D, E and F.

Some chemical analyses of the greasy rat skins showed that they contained noticeable quantities of fats and fatty acids with only a small amount of cholesterol. The total amount of fatty material was too minute to make further qualitative or quantitative determinations. Since normal skin seems to show a predominance of cholesterol and oxycholesterol as its fatty constituents, this might also indicate a disturbance in fat metabolism.

Discussion. In attempting to analyze these results, many things appear to be contradictory to the usual experience of nutrition workers. It has been commonly recognized that a ration containing 18 per cent casein and sufficient salts would produce a normal rate of growth when vitamins A and B were supplied by 5 per cent rendered butterfat and 0.6 gram of pure yeast given daily. When these conditions were duplicated in this study, the rats failed to grow normally and became thin and greasy.

The first suggestion of a possible cause might be that in using highly purified foods, 5 per cent butterfat no longer supplies a sufficient amount of vitamin A to promote growth. This supposition, however, fails to explain why the rats in groups I, II, III (fig. 1), with a smaller amount of vitamin A in their rations, grew at a better rate than the rats in group IV, receiving 5 per cent butterfat.

When whole butter was substituted for butter fat in a similar series of experiments, the vitamin A content of the ration was not increased, but a general improvement in growth, vitality and ability to reproduce was noted. This could only be attributed to the buttermilk fraction which is retained in whole butter. Evidently, the buttermilk was not able to entirely counterbalance the growth depressing influence, for the rats in group VII (fig. 1) receiving 5 per cent butter grew no better than those in group VI, receiving 2 per cent butter.

A possible deficiency of vitamin A is likewise not supported by the results with cod-liver oil. With 5 per cent cod-liver oil in the ration (group XIII, fig. 1), the growth depression was more noticeable than when 1 per cent or 2 per cent of the oil was present (groups XI and XII). Removing vitamin A from butterfat by hydrogenation threw no additional light on the subject.

A second possible explanation for this growth disturbance might be found in too low a level of vitamin B, even when supplied by yeast. When a vitamin B extract was obtained from wheat embryo, and fed in abundance, in addition to the vitamin A from 5, 10 and 15 per cent butterfat, respectively, the results were quite disconcerting, as failure to grow, emaciation and immaturity pointed to some heretofore unrecognized condition in the development of young animals. Since our basal diet, well supplied with both vitamins A and B continued to produce the same abnormal results, it appeared that the cause of the condition did not lie in an inadequate amount of these growth-promoting factors.

The next explanation which presented itself was that of some interrelationship of food materials or food factors, which might have a depressing effect upon the development and general health of young animals. Fridericia (16) has reported results showing that the blending of rendered lard or hydrogenated whale oil with butterfat has an inactivating action on the vitamin A in the butterfat: while vegetable oils, hydrogenated or non-hydrogenated, when mixed with butterfat at a low temperature, had no inactivating effect. In our experiments when butterfat was blended with lard and olive oil in varying proportions, and the results compared with those when butterfat, lard and olive oil were each used alone, the outcome was very unexpected. To find that 15 per cent lard in a ration produced the best growth and health in rats: that 15 per cent olive oil gave almost similar conditions and that 15 per cent butterfat yielded quite inferior animals, is difficult to interpret. The results from the use of blended fats also seemed to indicate that butterfat has some depressing effect on growth and metabolism when mixed with lard or olive oil, entirely contrary to former opinion. Butyric acid, one of the fatty acids peculiar to butterfat, did not prove to be the constituent of butterfat responsible for its unusual qualities (fig. 3).

With no conclusive evidence of interaction among fats, the next point considered was the relationship between the fat and the protein in the diet, from the suggestion made by Maignon (13). The four possible extreme conditions set up in the four special diets 1, 2, 3 and 4, involving fat and protein, each at a high and low level, gave some rather interesting results. When the fat was kept at a low level, the rats grew very well on a low level of protein, and not quite as well on a high level of protein; but when fat was kept at a high level, thin greasy rats were produced at both a low and high level of protein. This did not seem to indicate so much of an interrelationship between the fat and protein in the diet as a failure to utilize fat, thereby causing emaciation and greasiness. Smith and Carey (17) noted similarly that rats on a "high fat" diet failed to grow at a normal rate, but on a "high carbohydrate" diet (similar to group XXX, fig. 6), they grew somewhat above normal. They felt that possibly the imperfect

combustion of fatty acids might be responsible for subnormal growth rate, and that growth required the adjusting of fat and carbohydrate in an otherwise adequate diet.

Again, vitamins A and B may enter into some relationship productive of abnormalities in growth and maturity. Hartwell (18) in her studies of lactation, thinks that an excess of protein without adequate vitamin B causes ill effects. In figure 6, where all the rats were receiving ample supplies of vitamin B, better growth did occur in group XXX with low protein than in group XXXII, receiving high protein, as long as the fat intake was also kept at a low level: but when the fat was increased, no difference could be observed in the relative amounts of vitamin B and the protein occurring in the diet.

This seems to bring the problem back again to a question of fat utilization or fat metabolism. Takahashi (19) thinks that vitamin A is an important factor in fat metabolism and that only through that channel is it directly responsible for growth. Apparently, however, something else is necessary to complete the action, for this study has shown repeated failures in growth with an ample supply of both fat and vitamin A in the diet.

It is possible that in the preparation of our highly purified basal diet, we may have removed some compound which, when present, linked the fat with its vitamin into an intracellular lipin. Rosenbloom (20) believes that under normal conditions, fat is combined in a "masked" form,—a calcium-protein-fat compound, produced either by a mechanical mixture, a chemical union or an adsorption process. Cramer (21) similarly describes a "lipoid gland tissue" in rats, where he thinks that fat and vitamins are deposited. If the compound causing this linkage should be absent, the fat would not go into its "masked" form, but would remain free in the system. Vitamin A, not being linked with the fat, would also be in an unnatural condition, and might show an antagonistic attitude toward vitamin B.

On the basis of such an assumption we could explain the poor growth with 5 per cent butterfat and the better growth with whole butter, since the latter is not so pure as the butterfat. The inferior growth with 5 per cent cod-liver oil, as compared with the excellent growth on 1 per cent and 2 per cent cod-liver oil, would also seem reasonable. The ability of lard and olive oil to produce better results than butterfat and cod-liver oil would be explained by the fact that, since these fats carry little if any vitamin A, there would be no reaction with vitamin B. The superior quality of spinach oil compared with cod-liver oil as a protective agent in the feeding experiments, might be attributed to the presence, in addition to vitamin A, of a small amount of this unknown compound, which was able to link the fat and its accompanying vitamin into a form more usable for the cell. The poorer growth resulting from additional calcium in the ration might be due to its failure to link with fat, thus becoming a burden to the system.

The appearance of the greasy condition in the skin and hair of the rats would point to free fat in the system and not the usual "masked" form. The appearance of fat or fatty acids in the urine, as shown by the presence of Sudan III, would also indicate a surplus of these substances in the blood stream. Rosenbloom (20) mentions that under pathological conditions, free fat is found in the organs of the body. This is supported by chemical analysis of the greasy rat skins, which showed more fat and fatty acids than cholesterol; and by the appearance of the vertical sections of greasy and normal rat skins.

It may readily be seen that this work is only introductory in nature. However, it suggests some problems for further investigation in a new field. McCollum (22) in a recent paper indicates that he is beginning to realize that there are some unrecognized interrelationships that exist among the food materials. Evidently, the more highly purified we make these food materials, the more quickly will we come to know the factors and interrelationships, which help to promote normal growth and general good health.

CONCLUSION

1. When presumably adequate rations, made of highly purified food materials and including standard amounts of protein, salts and vitamins A and B, are fed to experimental rats, they fail to grow at a normal rate, remain sexually immature and often develop greasy skin and hair.

2. These abnormalities are not caused by too low a level of vitamins A and B.

3. Experimental data indicate that faulty fat metabolism or utilization is responsible for these abnormalities.

4. It is assumed that in the use of highly purified foods, some compound has been removed, which is essential for the utilization of fat by the body.

5. There are some indications of important interrelationships between the carriers of vitamins A and B.

Grateful acknowledgment is extended to Dr. C. M. Jackson, Professor of Anatomy, University of Minnesota, for his kindness in preparing the microphotographs used in this paper.

BIBLIOGRAPHY

(1) MacLeod, J. M. H. 1904. Practitioner, lxxii, 755.
(2) Stelwagon, H. W. 1916. Diseases of the skin. 8th ed., 1071. W. B. Saunders & Co., Philadelphia.
(3) Pusey, W. A. 1920. The principles and practice of dermatology. 3rd ed., 1122. D. Appleton & Co., New York.
(4) Sutton, R. L. 1923. Diseases of the skin. 5th ed., 984. Mosby Medical Book & Pub. Co., St. Louis.

(5) SABOURAUD, R. 1904. La Presse Med., nos. 45 and 50.
(6) SCHOLTZ, M. 1924. Med. Journ. and Record, cxix, 334.
(7) MESKA, A. 1924. Dermat. Wochenschr., lxxviii, 552.
(8) LE GENDRE, P. 1921. Nouveau Traite de Medecine. Fasc. vii. Avitaminosis.
(9) FRANK, A. 1923. Monatsch f. Kinderheilkunde, xxv, 147.
(10) GIVENS, M. H. AND L. B. MENDEL. 1917. Journ. Biol. Chem., xxxi, 421.
(11) HICKMANS, E. M. 1924. Biochem. Journ., xviii, 925.
(12) HOLT, L. E. AND H. L. FALES. 1922. Amer. Journ. Dis. Children, xxiii, 472.
(13) MAIGNON, F. 1919. Compt. Rend., lxxxii, 400.
(14) READ, B. E. 1919. Journ. Biol. Chem., xxxvii, 121.
(15) GAGE, S. H. AND P. A. FISH. 1924. Amer. Journ. Anat., xxxiv, 1.
(16) FRIDERICIA, L. S. 1924. Journ. Biol. Chem., lxii, 471.
(17) SMITH, A. H. AND E. CAREY. 1923. Journ. Biol. Chem., lviii, 425.
(18) HARTWELL, G. A. 1924. Biochem. Journ., xviii, 785.
(19) TAKAHASHI, K. 1924. Journ. Chem. Soc. (Japan), xliii, 201.
(20) ROSENBLOOM, J. 1911. Biochem. Bull., i, 75.
(21) CRAMER, W. 1920. Journ. Physiol., liv, ii.
(22) MCCOLLUM, E. V., N. SIMMONDS AND J. E. BECKER. 1925. Journ. Biol. Chem., lxiv, 161.

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